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Relationship between gene mutation and lung cancer metastasis

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Abstract Cancer is a genetic disease occurring through a multi-step process. Many important genes responsible for the genesis of various cancers have been discovered, their mutations precisely identified and the pathways through which they act characterized. One question that remains unanswered is whether the development of new, more specific therapeutic agents is the best way to minimize cancer morbidity and mortality in the long-term. Metastasis is the relentless pursuit of cancer to escape its primary site and colonize distant organs. Phenotypic changes during cancer progression reflect the sequential accumulation of genetic alterations, which endow cancer cells with the ability to undergo their own divergent evolution and create distinct metastatic species. In order to understand this process, it is crucial to identify genes whose alterations accumulate during cancer progression and correlate with metastatic phenotypes of cancer cells.

Keywords Non-small cell lung cancer (NSCLC) · Adenocarcinoma · KRAS · EGFR · Metastasis

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1 Introduction

Metastasis is not the biologic narrative of an autonomous cell but rather a dynamic interplay between neoplastic cells and newly encountered microenvironments [1]. Non-small cell lung cancer (NSCLC) currently remains the leading cause of cancer-related death worldwide; more than 200,000 new cases are diagnosed annually and only 16 % of patients survive longer than 5 years [2]. Lung adenocarcinomas contain characteristic, mutually exclusive mutations in receptor tyrosine kinase (RTK) or RAS pathway oncogenes, including frequent mutations of KRAS or EGFR, while translocations involving EML4 and ALK, RET, or ROS1 are also detected, though at lower frequencies [3]. Although the application of conventional and targeted chemotherapeutics today is widespread in clinical oncology, often succeeding in reducing tumor burden and improving survival, patients commonly progress to a refractory state. Genetic mutations, interactions with the microenvironment, and cancer stem cells are well-described mechanisms that contribute to disease progression and may explain why some tumor cells survive chemotherapy exposure, whereas others perish [4].

In the past, metastatic spread was considered a late event occurring long after primary tumors had progressed locally, which may still be true for some tumors. However, recent evidence suggests parallel progression of primary and metastatic disease with circulating tumor cells are detected even in newly diagnosed early-stage cancer [5]. Several developmental pathways, such as the Hedgehog (Hh) signal transduction cascade, the Wnt/β-catenin pathway, or the transforming growth factor-β (TGFβ) and the Notch pathway, have been identified as endowing cancer cells with the capacity to become resistant to treatment, self-renew, and metastasize [4]. In tumors like clear cell renal carcinoma, intratumor heterogeneity may have an impact on the strategy when taking biopsies in

order to have a full picture of the disease mutational landscape and the malignant evolutionary process of metastases [6, 7]. However, this does not seem to be the case for lung adenocarcinomas, where a single-biopsy analysis at an appropriate depth might be sufficient to identify the majority of known cancer gene mutations [8].

In this short, groundbreaking review, we will try to comprehensively cover novel and underappreciated genetic alterations, which result in uncontrolled lung cancer cell growth and metastatic disease.

2 Existing knowledge of lung cancer metastasis

Compared with other common primary tumors, such as colorectal and breast cancer, where median survival is more than 20 months, the prognosis of metastatic NSCLC remains extremely poor. The survival of NSCLC patients can range from 10 to 60 % in stage II and stage IIIA with the risk of relapse being rather unpredictable despite the many prognostic gene signatures that have been developed [9]. In completely resected NSCLC, disease relapse rates remain high. Adjuvant chemotherapy after complete resection has been shown to improve survival in patients with NSCLC [10, 11]. The requirement for molecular markers to identify, for example, stage II patients with good prognosis who could be spared chemotherapy and high-risk stage IA patients who might need chemotherapy is an important and as yet unmet medical need [12].

Two large meta-analyses of adjuvant and preoperative chemotherapy in early resected NSCLC show an absolute increase in survival of 4 % at 5 years [10, 11]. Neither meta-analysis showed that age, histology, or clinical stage could predict benefit from adjuvant or preoperative chemotherapy [10, 11]. However, in the adjuvant chemotherapy meta-analysis, there was a trend toward a negative effect of chemotherapy in the small subgroup of stage IA patients [10]. A 14 gene expression assay using quantitative PCR was able to discriminate the risk of recurrence in stage I non-squamous NSCLC with 5-year overall survival (OS) of 71.4 % in low risk, 58.3 % in intermediate risk, and 49.2 % in high-risk patients [13]. Genes in this prognostic signature, including BRCA1 and YAP1, are central to crucial oncogenic pathways [13, 14]. Acquired resistance to KRAS suppression in a KRAS-driven murine lung cancer model involves increased YAP1 signaling. KRAS and YAP1 converse on the transcription factor FOS and activate a transcriptional program involved in regulating the epithelial-mesenchymal transition (EMT) [15]. Therefore, among the different gene signature models, there are still many inconsistencies.

Metastasis is responsible for a large morbidity and mortality burden among cancer patients; currently, however, few therapies specifically target metastatic disease. As with breast cancer, bone and lung metastases are particularly frequent in NSCLC. However, the different distribution patterns of metastases in lung carcinoma are poorly understood, probably because studies are difficult, given the extremely short survival times and high proportion of patients who have widespread metastases at the time of diagnosis. Further scientific dissection of the underlying pathways is required to pave the way for new therapeutic targets.

Metastases can be seen in the contralateral lung in 26–28 %, in bone in 35–43 %, in liver in 18–20 %, and in adrenals in 21–27 % of patients [16]. In the study of Crawford *et al.*, bone metastases together with performance status were found to be an independent prognostic variable [16]. The most comprehensive analysis of the pattern of metastases is based on the pooled data of 1436 patients with metastatic NSCLC who were treated in two clinical trials [17, 18]. Ipsilateral lung metastases were recorded in 67 %, contralateral lung metastases in 35 %, bone metastases in 35 %, liver metastases in 22 %, pleural involvement in 32 %, brain metastases in 10 %, supraclavical nodal involvement in 14 %, subcutaneous metastases in 4 %, mediastinum metastases in 53 %, and metastases in other organs in 32 % of patients. Subcutaneous and liver metastases and more than four metastatic sites were identified as independent markers of poor prognosis [19]. The International Association for the Study of Lung Cancer's (IASLC) International Staging Project on Lung Cancer evaluated 6596 metastatic NSCLC patients for survival according to the distribution of metastases. Median survival was 13 months for patients with ipsilateral lung metastases, 10 months for those with contralateral lung metastases, 8 months for those with pleural dissemination, and 6 months for those with other distant metastases [20]. Given that the basic genomic program of the primary tumor may predetermine its metastatic capability, careful examining the expression of putative metastasis-associated genes in the primary tumor may help to identify subclinical micrometastases, predict risk of recurrent disease, and guide selection of patients who might benefit from adjuvant chemotherapy.

NSCLC is now recognized as a heterogeneous set of diseases. In patients with lung adenocarcinomas, epidermal growth factor receptor (EGFR) mutations are associated with response to EGFR tyrosine kinase inhibitors (TKIs) [21, 22]. Other potentially targetable oncogenes are HER2, MET, FGFR1, and KRAS, as well as fusion oncogenes involving anaplastic lymphoma kinase (ALK), ROS1, neureregulin 1 (NGR1), and neurotrophic tyrosine kinase receptor 1 (NTRK1). Potentially targetable mutations have also been identified in squamous cell carcinoma of the lung, such as discoidin domain-containing receptor 1 (DDR2), FGFR1, and others [21, 22]. Therefore, in most cases, adjuvant chemotherapy may not be the correct strategy and could even have a nefarious effect.

3 Novel and underappreciated molecular pathways involved in lung cancer metastasis

EGFR mutations are an early event in the development of NSCLC, and heterogeneous distribution of an *EGFR* mutation between primary and metastatic lesions is not very common [23]. In contrast, *EGFR gene* amplification is more frequent in metastatic lesions, possibly contributing to the development of a metastatic phenotype [23–25]. Although the EGFR pathway seems to play an important role in NSCLC metastasis, whether or not *EGFR* mutations are more frequent in NSCLC with brain metastasis is not clear [26]. The association of brain metastasis with *EGFR* amplification and human epidermal growth factor receptor 3 (HER3) overexpression has been demonstrated. Further studies to evaluate the potential role of *EGFR* overexpression or amplification as a predictive biomarker in NSCLC with brain metastasis could be of great relevance [26].

RAS-pathway mutant NSCLC cells depend on the transcription factor (GATA2) for their viability [27]. This dependency occurs *via* concurrent regulation of the proteasome machinery, the IL-1/NF-κB signaling pathway, and the Rho-signaling cascade [27]. *KRAS* and *EGFR* mutant cells, as well as cells with mutations in *NRAS*, *NF1*, and *EML4-ALK*, show a striking dependency on GATA2 for mutant cell viability [27]. In chronic myeloid leukemia, ecotropic viral integration site 1 (Evi1), an indispensable transcription factor in stem cells regulation, is a valuable prognostic marker of myeloid malignancies [28]. Evi1 has diverse functions as an oncoprotein, including suppression of TGFβ-mediated growth inhibition, negative regulation of the c-Jun N-terminal kinase pathway, and stimulation of cell growth by activator protein-1 (AP-1). Evi1 has also the potential to recruit and activate diverse proteins for transcriptional regulation, including GATA2 [29] (Fig. 1).

T lymphokine-activated killer cell-originated protein kinase (TOPK) expression is clinically associated with PTEN expression and can be used as an independent prognostic factor to predict the treatment outcomes of patients with lung cancer [30]. TOPK is a Ser/Thr protein kinase that is highly expressed in many types of human cancer, including breast and lung, but is undetectable in normal tissues, except the germ cells of testis and several fetal tissues [31]. TOPK was included in the "consensus stemness ranking signature" gene list that is upregulated in cancer stem cell-enriched tumors and is associated with poor prognosis in multiple types of cancer [32]. In an attempt to identify genes associated with metastasis in lung cancer, Shih *et al*. integrated the transcriptomes of lung adenocarcinoma and metastasis signature data sets and identified TOPK as a potential target [30]. Indeed, TOPK may stimulate AKT-dependent cell migration by relieving a PTEN-dependent suppressive effect to facilitate cancer metastasis [30] (Fig. 1). TOPK modulates the protein stability of PTEN by stimulating it to undergo proteasome-dependent degradation, inducing cell migration in a PI3K/AKT-dependent manner [30]. In the same study, it was found that high expression of TOPK is significantly associated with advanced stage and lymph node metastases and is a prognostic factor of both overall and disease-free survival for lung cancer patients [30]. Interestingly, the hazard ratios of overall and

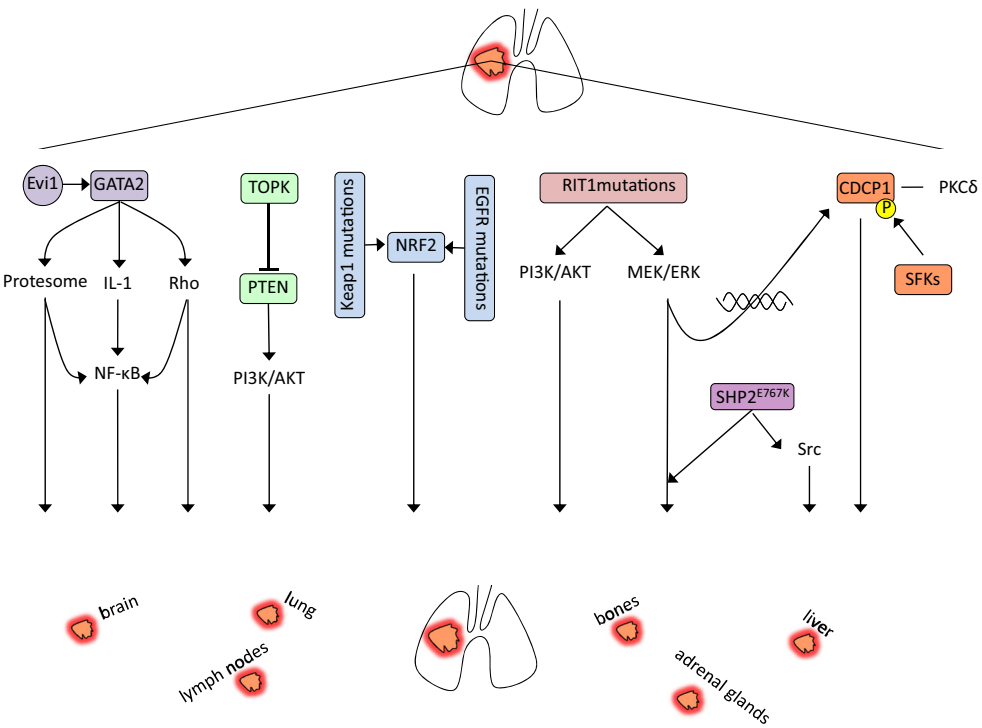

Fig. 1 Novel and underappreciated genetic alterations that can induce lung cancer metastasis

disease-free survivals for TOPK were higher than that of lymph node status and close to that of distal metastasis status [30]. Patients with high TOPK and low PTEN levels had a significantly poorer outcome in terms of overall and disease-free survivals compared with the group of patients with low TOPK and high PTEN levels. Therefore, TOPK is a promising molecular target for therapy and a prognostic marker for recurrent lung cancer. A liposomal formulation of OTS964, a TOPK inhibitor, has been shown to be a potent therapeutic agent that may be applied to a wide range of human malignancies [31].

The transcription factor NF-E2-related factor2 (Nrf2) is abundantly expressed in lung cancer cells and plays a pivotal role in NSCLC proliferation and chemoresistance [33]. It is a cap 'n' collar basic leucine-zipper transcription factor originally identified as a pivotal factor for cell protection from oxidative and electrophilic insults [33]. Nrf2 is dually regulated by an Nrf2 repressor protein Keap1 (Kelch-like ECH-associated protein-1) and EGFR signaling. NSCLC cells with wild-type EGFR and Keap1 can proliferate under EGFR ligand stimulation, while stress conditions such as exposure to cigarette smoke extract enhance cell proliferation by modifying the Nrf2/Keap1 interaction [33]. Exposure to cigarette smoke extract or knockdown of Keap1 mRNA can reduce EGFR TKI efficacy in EGFR mutant NSCLC [33]. Therefore, Nrf2, a downstream molecule of both EGFR and Keap1 signaling, can be an important molecular target for the treatment of NSCLC with EGFR, KRAS, or Keap1 mutations. At the same time, Keap1 dysfunction may become a novel molecular marker to predict resistance to EGFR-TKI in EGFR mutant NSCLC [33] (Fig. 1).

Somatic RIT1 mutations have been recently identified as novel driver mutations in lung adenocarcinoma [34]. RIT1 encodes a RAS-family small GTPase with significant domain and sequence homology to KRAS, HRAS, and NRAS, and intrinsic GTP hydrolysis activity [34]. Somatic mutations in this small GTPase gene, that cluster in a hotspot near the switch II domain of the protein, have been reported in almost 2 % of lung adenocarcinoma [34]. RIT1 mutations are mutually exclusive to all other known lung adenocarcinoma oncogenes and rapidly induce transformation *in vitro* and *in vivo* by inducing activation of PI3K and MEK signaling which can be reversed by combined PI3K and MEK inhibition [34] (Fig. 1).

SHP2 is a classical, non-receptor protein tyrosine phosphatase (PTP) encoded by the *PTPN11* gene [35]. Binding of the SHP2 SH2 domains to specific phosphotyrosine docking sites such as GAB1, in response to tyrosine kinase activation, induces SHP2 activation and RAS-ERK1/2 and Src downstream signaling [35]. Besides activation *via* binding of its SH2 domains to phosphotyrosine-based docking sites, gain-of-function SHP2 mutations have been described in human cancer, mainly in hematologic malignancies and, more rarely, in lung cancer (1.81 %). However, since lung cancer is a major lethal disease, a small percentage of mutations could represent a large number of affected patients and thus should not be ignored, especially mutations actionable for developing new-targeted therapies. In the study by Schneeberger *et al*., the $SHP2^{E76K}$ mutation activated ERK and Src and upregulated c-Myc and Mdm2 in the lungs of *in vivo* models. Mutant SHP2 promoted lung tumorigenesis and was required for tumor maintenance [35]. At the same time, Gab1 tyrosine phosphorylation was sensitive to inhibition by the Src inhibitor dasatinib in activating SHP2-mutant-expressing cells [35] (Fig. 1).

The significance of CUB-domain-containing protein 1 (CDCP1) is a regulator of metastatic aspects of progressed cancers under the control of the Ras and Src family kinases (SFKs) signal pathways. [36]. CDCP1, also known as SIMA135, gp140, and Trask, is a type I transmembrane protein that has possible roles in cell-cell and cell-matrix adhesion and is highly expressed in lung, breast, and colon cancers [37]. CDCP1 controls tumor cells' metastatic and invasive potential without significantly affecting proliferation and can be an ideal therapeutic target of metastatic cancers. Anoikis is a form of apoptosis triggered by the loss of cell survival signals generated from interaction with the extracellular matrix [38]. Resistance to anoikis, acquired during carcinogenesis, has been described as a core aspect of cancer cells for tumor progression and metastasis [39]. Several studies have shown the crucial role of SFKs in tumor cell anoikis resistance [40, 41]. CDCP1 was identified as a key molecule of anoikis resistance in lung adenocarcinoma, mediating signals from activated SFKs in human cancer cells; it is actually a modulator of the later processes of cancer metastasis through the regulation of anoikis [37]. The CDCP1– protein kinase Cδ (PKCδ) pathway is required *in vivo* for distant metastasis of lung cancer cells in a mouse model [37]. CDCP1 is tyrosine phosphorylated by activated SFKs and directly binds to PKCδ [38]. Phosphorylated CDCP1 recruits PKCδ to the plasma membrane causing activation of this molecule, which results in the acquirement of anoikis resistance, enhanced cell migration and invasion, and metalloproteinase secretion *in vitro*. The CDCP1 pathway does not significantly support cell growth or tumorigenicity, whereas it clearly regulates tumor metastasis [37]. Recent reports also suggest that cleavage of CDCP1 at the extracellular domain to produce 70-kDa form might have a role in the activation of CDCP1 signaling [42]. Ikeda *et al*. examined expression of CDCP1 in 200 stage I–III lung adenocarcinoma patients by immunohistochemistry [43]. Significant positive correlation was observed between CDCP1-high expression and relapse rate, poor prognosis, and occurrence of lymph node metastasis [43]. Very recently, it was demonstrated that expression and tyrosine phosphorylation of CDCP1 by Ras and SFKs, respectively, are essential for acquisition of the metastatic and invasive properties of cancer cells [36]. The activity of ERK downstream of K-Ras

regulates the expression of CDCP1. Part of the malignant characteristics induced by activated Ras, such as anoikis resistance, migration, and invasion, are dependent upon the upregulation of CDCP1 protein. Oncogenic Ras promotes both proliferative and metastatic potential of cancers, but induction of CDCP1 is only responsible for the metastatic potential induced by Ras [36]. The utilization of CDCP1 as a target molecule will be especially advantageous when combined with conventional antiproliferative drugs for the treatment of progressed tumors (Fig. 1).

4 Conclusions

It is clear that multiple pathways, including both those promoting and suppressing tumor growth, can be altered, facilitating the genesis and progression of NSCLC. It is also clear that targeting activating mutations and their downstream biochemical pathways is easier and more practical for developing novel therapeutics. New data derived from genome-wide screening efforts, deep sequencing, and large-scale gene expression profiling will provide additional insights into potential molecular targets that can be manipulated for therapeutic purposes. The success of such efforts will lead to improvements in the prognosis and quality of life of NSCLC patients.

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Conflict of interest The authors declare that they have no conflict of interest.

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