OncoTargets and Therapy

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REVIEW

Rationale for Lung Adenocarcinoma Prevention and Drug Development Based on Molecular Biology During Carcinogenesis

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Lung adenocarcinoma (LUAD) is the most common and aggressive subtype of lung cancer with the greatest heterogeneity and aggression. Inspite of recent years' achievements in understanding the pathogenesis of this disease, as well as the development of new therapeutic approaches, our knowledge on crucial early molecular events during its development is still rudimentary. Recent classification and grading of LUAD has postulated that LUAD does not arise spontaneously, but through a stepwise process from lung adenomatous premalignancy atypical adenomatous hyperplasia to adenocarcinoma in situ, minimally invasive adenocarcinoma, and eventually frankly invasive predominant adenocarcinoma. In this review, we discuss the molecular processes that drive the evolutionary process that results in the formation of LUAD. We also describe how to handle lung premalignancy in clinical settings based on the most recent advances in genomic biology and our own understanding of lung cancer prevention.

Keywords: lung adenocarcinoma, molecular biology, pathogenesis, cancer prevention

Introduction

Lung cancer is the predominant cause of cancer deaths worldwide.[1,2] Most patients with lung cancer present with advanced-stage disease when diagnosed, and even in patients with early-stage resectable or locally advanced disease receiving definitive chemo- or radiation therapy, up to 90% of will still inevitably have disease recurrence, with a 5-year survival rate <60%.[2,3] Non-small-cell lung cancer (NSCLC) represents 80%–85% of lung cancers, and can be subdivided into adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma.[4] Among all NSCLC subtypes, lung adenocarcinoma (LUAD) is the most heterogeneous and aggressive and has a very high tumor-mutation burden associated with *EGFR*, *KRAS*, *BRAF*, *ERBB2*, *TP53*, *ALK*, *STK11*, and *TTE1* mutations.[5–8] Despite advances in chemotherapy, radiation therapy, and targeted therapy in the last decade, prevention and early detection and treatment of lung cancer is still challenging, due to limited awareness of molecular mechanisms mediating early lung carcinogenesis and also the very late diagnosis of the majority of them.[9]

The intriguing development of next-generation sequencing in recent years has defined a large number of driver mutations of cancer, leading to malignancy-therapy advances.[10] The establishment of the Pre-Cancer Genome Atlas, a project for characterization of molecular evolutions from premalignant lesions to invasive carcinoma, as well as corresponding changes in the tumor microenvironment (TME) in 2016, has also helped to

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http://doi.org/10.2147/OTT.S248436

provide us the insight to consider another direction for cancer treatment, ie, cancer prevention.[11] A complex and stepwise process from the only known type of preneoplasia, atypical adenomatous hyperplasia (AAH), to a more pronounced cellular atypia adenocarcinoma in situ (AIS) to microinvasive lesion minimally invasive adenocarcinoma (MIA), and finally to LUAD involving several genetic and epigenetic alterations has been postulated based on a variety of pathological, molecular, and clinical studies.[6,12–14] In this review, we set out an organizing framework for understanding molecular biology in LUAD premalignancy, in order to set out a theoretical background for cancer prevention–drug development. In addition, we highlight possible intervention approaches that might prevent progression of premalignant lesion to LUAD.

Pathological and Molecular Alterations During Lung Carcinogenesis

Our lungs are continuously exposed to the outside environment, and thus harbor a complex network protecting the host from tissue damage and infection.[15,16] The multistage stepwise fashion of tumor development has been demonstrated in various anatomical organs.[17,18] Therefore, a paradigm referred to as "field cancerization" has been created for areas of histologically normal-appearing tissue that exhibit molecular abnormalities during early tumorigenesis.[17,19] Besides cigarette smoking, environmental exposure, such as air pollution and workplace exposure to asbestos, diesel exhaust, or certain chemicals, also creates a field of injury in airway epithelial cells: dysregulated repair by progenitor cells forming a clonal group of indefinitely self-renewing daughter cells in the initial phase, and proliferation and expansion of premalignant cells resulting from genetic and epigenetic alterations gradually displacing the normal epithelium.[20–24]

AAH and AIS: Field Cancerization

Lung preneoplastic-lesion AAH and preinvasive-lesion AIS present cellular atypia characteristic of field cancerization.[17,20,25,26] As the only known type of preneoplastic lesion, AAH represents the initial step in LUAD pathogenesis.[13,14,27–29] It is a small, atypical proliferation (usually 0.5 cm or less) of type II pneumocytes along preexisting alveolar walls most commonly discovered as an incidental histological finding in 5%–20% of lung cancer specimens after resection.[12,27,30,31] Telomere attrition has been demonstrated to occur as an initiating event in AAH progression to LUAD.[32] An intriguing finding is that shared mutations of two classical genes, *KRAS* and *EGFR*, of AAH and LUAD in the same individual have also been detected,[33–37] though Sakamoto et al[38] showed that harboring a *KRAS* gene mutation might not ensure AAH's further progress to LUAD. Other molecular aberrations identified in AAH include *FGFR3*,[39] *BRAF*,[40] *TP53*,[29,41] *STK11*,[42] and *ERBB2* (*HER2*) mutations,[43] upregulation of cyclin D1,[44] survivin,[45] paxillin,[46] and Ki67,[47] suppression of TBX2,[48] and loss of heterozygosity in chromosomes 3p, 9p, 9q, 16p, 17p and 17q.[29,49–52] Epigenetic modifications including DNA methylation of CDKN2A-Ex2 and PTPRN2 have also been reported in AAH.[25,53] Genes bearing somatic mutations or epigenetic modifications often encode tumor-associated antigensable to elicit immunoresponse; therefore, the immune system is capable of recognizing AAH. T-effector and cytotoxic cell infiltration and upregulation of immunocheckpoint PDL1 and CTLA4 in AAH compared to normal lung tissue suggest that the T cells might have already been activated in the AAH stage.[40,54] Activation of protumor (T_H2; *CCR2*, *CTLA4*) and reduction in antitumor (T_H1; *IL12A*, *GZMB*, *TBX21*) immunofunctioning-associated gene sets observed in the development from normal lung to AAH further confirm the involvement of aberrant immunopathways in AAH.[55]

Growth and progression of AAH with a more pronounced cellular atypia in morphology evolve into AIS, a small localized (≤3 cm) adenocarcinoma with restricted growth of neoplastic cells along preexisting alveolar structures.[56] AIS is also regarded as a preinvasive form of LUAD according to recent classification and grading.[28] Progression from AAH to AIS occurs over an extended period, with different alterations detected. A recent study by Izumchenko et al[57] indicated that only a few gene mutations were shared between AAH and AIS, and that the most frequent base-pair substitutions were C–T transitions in AAH, but G–A in MIA. Tanaka et al[58] showed that *BI1* is expressed in AIS, but not in AAH. Chung et al[59] combined AAH and AIS as preinvasive lesions and investigated their multistep progression to adenocarcinoma. Methylation of *HOXA1*, *TMEFF2*, and *RARB* was frequently observed in preinvasive lesions, suggesting epigenetic alterations in different genes involved in different stages.

Invasive Lesions: MIA and Adenocarcinoma

Malignant cells become invasive once they leave the epidermis.[60–62] These invasive lesions generally harbor driver mutations, including *EGFR*, *KRAS*, *ALK*, *ERBB2*,

BRAF, *AKT1*, *PIK3CA*, *MAP2K1*, and *MET* mutations;[63,64] however, few of these have been reported in precursor lesions.[33–43] A subset of invasive adenomatous lesions appears to arise spontaneously in the absence of any precursor lesions for only around 15% of LUAD-harboring precursor lesions,[65] though it may also be possible that the precursors are no longer detectable at diagnosis.

Substantive changes occur during the evolution to invasive lesions from MIA and adenocarcinoma from preinvasive lesions. In contrast to AAH and AIS, microinvasion is present in MIA, small, solitary, and discrete microinvasive lesions no larger than 3 cm with a predominantly lepidic pattern and invasion <5 mm in any one focus, the majority of which are nonmucinous.[6,14] Currently, genomic studies into MIA have been limited compared to preneoplastic lesions, though unsurprisingly a higher frequency of *KRAS*, *EGFR*, *TP53*, and *NF1* mutations has been found in MIA than AAH and AIS.[57,66] A recent study by Qian et al[66] also demonstrated an increasing frequency in mutations of *KRAS*, *TP53*, and *NF1* in MIA than its earlier stage — AIS.

Once the invasive area of MIA extends >5 mm in diameter or meets invasion criteria, the lesion becomes an invasive predominant adenocarcinoma.[6,14] The mutational landscape of early-stage LUAD has been investigated by multiple studies: *EGFR*, *TP53*, *KRAS*, *STK11*, and *NF1* are substantially mutated in LUAD, and driver mutations in *EGFR*, *BRAF*, *MET*, and *TP53* are almost always clonal, with acquired mutations directly linked to patient prognosis.[8,13,67] It has also been established that mutations acquired during progression are linked to patient prognosis.[13] With acquisition of more mutations, the immune system also becomes more complicated in early-stage LUAD. An example is that inactivation of *STK11* will result in accumulation of immunosuppressive neutrophils and reduced PDL1 expression, as well as fewer tumor-infiltrating lymphocytes.[68]

Rationale for LUAD Prevention: A Clinical Insight

In clinical practice, preinvasive and early invasive LUAD lesions can be detected by computed tomography or during histopathological studies on surgically resected specimens, and present as pulmonary nodules with ground-glass opacity (GGO): circumscribed hazy lesions with preservation of bronchial and vascular margins.[9,69] Prediction of GGO nodules is difficult, as a considerable proportion will disappear spontaneously; however, approximately 10% will progress to invasive cancer.[70–72] Therefore, management of incidentally detected GGO nodules is recommended as a follow-up for a minimum 3–4 years.[73,74] According to the American College of Chest Physicians, increased size or solid-component development, pure GGO nodules >10 mm with confirmed persistence, mixed (GGO >50%) GGO nodules >8 mm with confirmed persistence, or mixed GGO nodules >15 mm without follow-up should be considered for surgery.[75,76] However, patients may still relapse following resection: according to a report from Cho et al[77] 5.1% (five of 97) of patients with GGO nodules experienced recurrence after resection, while Nakao et al reported that 8% (four of 50) of patients with GGO nodules recurred after limited section.[78] For patients with early-stage NSCLC following standard treatment surgery, as many as 40% of patients with stage I and 66% of stage II NSCLC are still found to relapse and finally die within 5 years.[79–82] In addition, surgical resection may not be feasible for patients carrying multiple potentially aggressive transformation nodules.[83] Therefore, drug development for lung cancer prevention and interception becomes our next-step consideration. Gene mutations can lead to activation of specific oncogenic pathways, leading to the occurrence of cancer. Therefore, targeting key molecular events will help in lung cancer interception (Figure 1). Molecular changes also have major effects on the TME, and thus secretion of specific inhibitory cytokines or production of chemokines and other factors related to immunosuppression. A typical example is that early *KRAS*-mutated pancreatic neoplastic cells can secrete VEGF, GM-CSF, and cytokines to recruit T_{reg}s, myeloid-derived suppressor cells, adipocytes, neutrophils, macrophage, and chemokines, leading to a progressively immunosuppressive TME that contributes to immunoescape.[84,85] In NSCLC, *EGFR* and *STK11* mutations are more likely to have low levels of PDL1 expression and mutational burden, thus lacking benefit in immunoheckpoint blockadetherapy.[86–90] As to the positivity of $EGFR^{T790M}$, Haratani et al[91] showed that as a result of higher PDL1 expression level in $EGFR^{T790M}$-negative patients, they are more likely to benefit from nivolumab after EGFR TKI treatment. A recent study by Hastings et al[92] suggested that *EGFR*-mutant tumors have generally low response to immunocheckpoint inhibitors, but outcomes varied by allele, eg, lung tumors with $EGFR^{\Delta 19}$ alterations harbored a lower tumor-mutation burden compared with $EGFR^{L858R}$ lung tumors. In contrast, *TP53* and *KRAS* mutations and loss of PTEN and STK11 were observed to increase with PDL1 expression and mutational burden, as well as activated T-effector and IFNγ

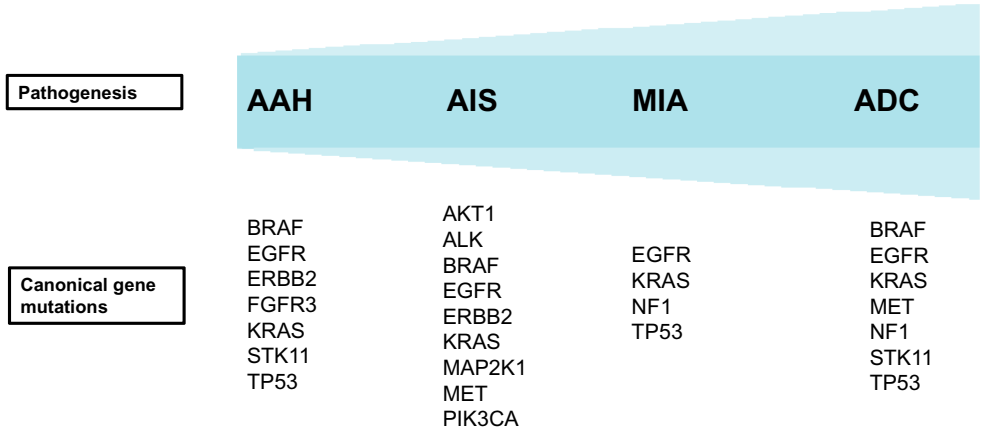

Figure 1 Summary of genetic alterations in multistep progression of lung adenocarcinoma.
Abbreviations: AAH, atypical adenomatous hyperplasia; AIS, aAdenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; ADC, adenocarcinoma.

signature in LUAD, and thus remarkable clinical benefit to PD1 inhibitors are found in patients with *TP53* and *KRAS* mutations.[7,68,93,94] Therefore, TME interception could also be a potential interception method.

Conclusion

As the most common subtype of lung cancer, LUAD has gained great attention from oncologists. In this review, based on the most recent International Association for the Study of Lung Cancer–American Thoracic Society–European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma, we elaborated a conceptual framework to reveal the stepwise genomic evolution of LUAD from preinvasive AAH and AIS lesions to invasive MIA lesions and adenocarcinoma. We also proposed a scheme for LUAD prevention based on our clinical perspective.

The advent of sequencing technologies has allowed us to understand pathogenesis during LUAD development; however our knowledge of its detailed pathogenesis remains somewhat superficial. Firstly, the current treatment method for pulmonary nodules with GGO in the clinic is observation and a wait-and-see approach once they are detected by computed tomography scan. Associations between antigen expression and the mechanism of cellular transformation are unknown. In addition, how long GGO nodules should be followed up, appropriate timing for surgical resection, and the extent of resection are other questions.[95] Secondly, many risk factors contribute to the development of LUAD, and recognizing these risk factors can be a potential consideration for prevention. As we all know, human papillomavirus vaccination has already been successfully used in cervical cancer. However, many gene-modified autologous vaccines for LUAD have already been tested, but not with the effects we expected.[10,96] Thirdly, alterations in the TME during carcinogenesis, especially immuneffector processes mediating cancer elimination, equilibrium, and escape, need to be elucidated. Potential interception of LUAD development would be enhancing the immune system's recognition and elimination or weakening immuno escape of the abnormal preneoplasias, ie, immunoprevention. Therefore, identifying molecular mechanisms and antigen expression in premalignancy responsible for immunoediting becomes crucial. Last but not least, beyond what we have summarized, other challenges associated need to be addressed, includingmaintenance of the lung environment, key driving events, and personalized cancer intervention.

Overall, this review serves to enrich our knowledge of molecular aspects ofLUAD pathogenesis. A limitation of this review is that we did not explicitly address the concrete genomic evolution during early lung adenomatous progression due to current superficial understanding, but we believe that our viewpoints will help to propel precancer research in the next few years and eventually expand cancer prevention to a greater subset of patients.

Disclosure

The authors have declared that no competing interest exists in this work.

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