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REVIEW

Pulmonary squamous cell carcinoma and lymphoepithelial carcinoma – morphology, molecular characteristics and differential diagnosis

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Pulmonary squamous cell carcinoma and lymphoepithelial carcinoma – morphology, molecular characteristics and differential diagnosis

Squamous cell carcinoma (SCC) comprises one of the major groups of non-small-cell carcinoma of the lung, and is subtyped into keratinising, non-keratinising and basaloid SCC. SCC can readily be diagnosed using histomorphology alone in keratinising SCC. Confirmatory immunohistochemical analyses should always be applied in non-keratinising and basaloid tumours to exclude differential diagnoses, most prominently adenocarcinoma and high-grade neuroendocrine carcinoma, which may have important therapeutic consequences. According to the World Health Organisation (WHO) classification 2015, the diagnosis of SCC can be rendered in resections of morphologically ambiguous tumours with squamous immunophenotype. In biopsies and cytology preparations in the same setting the current guidelines propose a diagnosis of 'non-small-cell carcinoma, favour SCC' in TTF1-negative and p40-positive tumours to acknowledge a possible sampling bias and restrict extended immunohistochemical evaluation in order to preserve tissue for molecular testing. Most SCC feature a molecular '-tobacco-smoke signature' with enrichment in GG > TT mutations, in line with the strong epidemiological association of SCC with smoking. Targetable mutations are extremely rare but they do occur, in particular in younger and non- or light-smoking patients, warranting molecular investigations. Lymphoepithelial carcinoma (LEC) is a poorly differentiated SCC with a syncytial growth pattern and a usually prominent lymphoplasmacytic infiltrate and frequent Epstein–Barr virus (EBV) association. In this review, we describe the morphological and molecular characteristics of SCC and LEC and discuss the most pertinent differential diagnoses.

Keywords: lung cancer, lymphoepithelial carcinoma, NSCLC, pathology, squamous cell carcinoma

Introduction

Pulmonary squamous cell carcinoma (SCC) and pulmonary lymphoepithelial carcinoma (LEC) are both non-small-cell lung carcinomas (NSCLC) with squamous differentiation.[1] SCC is the second most common histological type of NSCLC (after adenocarcinoma), accounting for approximately 20% of lung cancers. In the vast majority of patients, SCC is associated with smoking. Although classically a centrally located tumour, peripheral-type SCCs have been described and now account for approximately one-third of lung SCCs.[2–4] These occur more commonly in older patients and females, and may occur in association with fibrotic interstitial lung disease.[5] They

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generally present with lower pathological stage.[2] Peripheral-type SCCs are not formally classified as a subtype according to the World Health Organisation (WHO) classification, and there are no known specific histological or molecular features.[1]

LEC is a poorly differentiated SCC admixed with a variable but often brisk lymphoplasmacytic infiltrate, frequently associated with Epstein–Barr virus (EBV). These are rare tumours, accounting for fewer than 1% of NSCLC. They present predominantly in younger, non-smoking patients of Asian origin, with a median age of 51 years (range = 9–74 years).[1] It is in patients of Asian origin that the association of LEC with EBV is most pronounced (> 90%). Conversely, LEC in Caucasian patients is significantly less frequently associated with EBV infection.[1]

SCC and LEC are both staged according to the tumour–node–metastasis (TNM) classification system, currently available in its 8th edition.[6] While LECs are often coin lesions with pushing borders, conventional SCC is usually poorly delimited and sometimes centrally cavitated. SCC may rarely present as a superficial spreading tumour with an invasive component limited to the bronchial wall. It is then staged pT1a, irrespective of the size of the lesion. Metastases occur most frequently in hilar or mediastinal lymph nodes, followed by distant metastases to the bone, brain, lung and pleura, liver and adrenal glands, listed in decreasing order of frequency.[7]

In the following, we provide the reader with an in-depth review of the morphological and molecular characteristics of SCC and LEC and discuss the most pertinent differential diagnoses.

Histomorphological features of SCC

One of the novelties of the current WHO classification 2021 has been the introduction of essential and desirable criteria for each diagnostic entity.[1] The WHO essential criteria to diagnose SCC are the presence of keratinisation and/or intercellular bridges (Figure 1) and/or the expression of immunohistochemical markers of squamous differentiation. SCC is

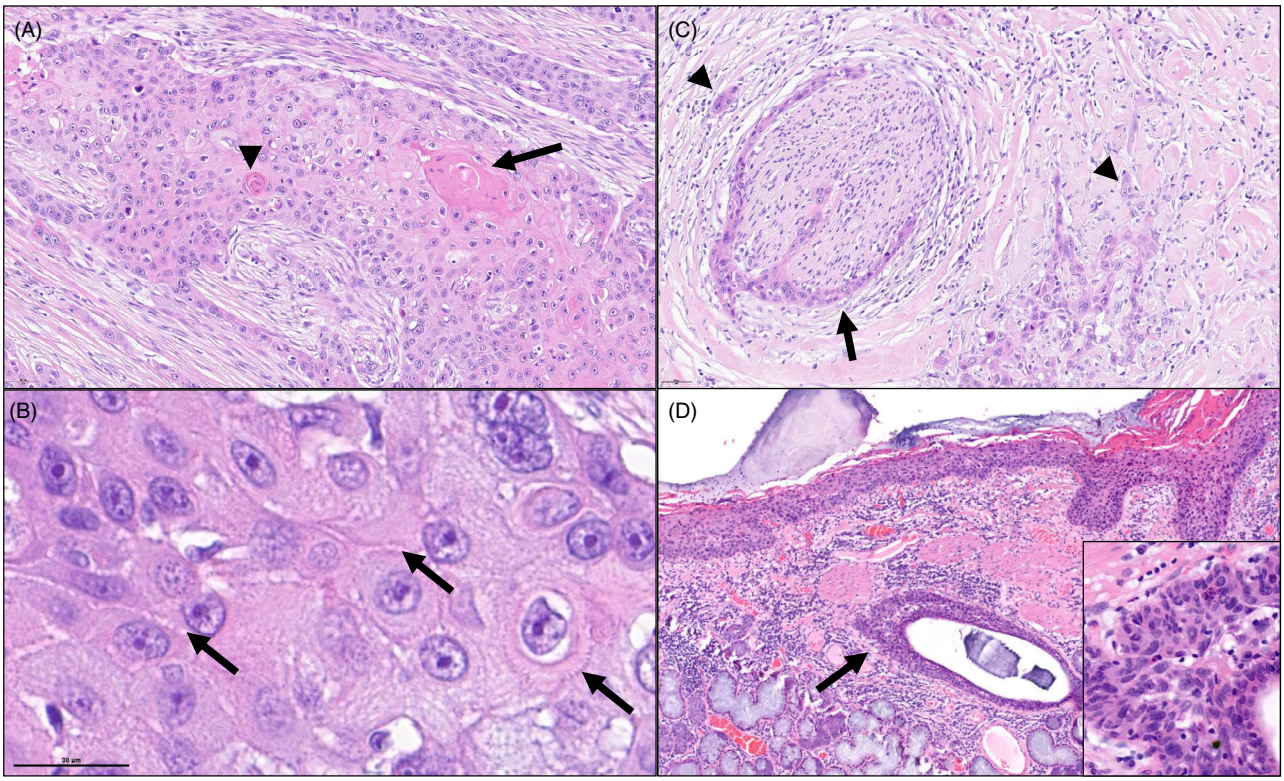

Figure 1. Keratinising squamous cell carcinoma. **A,** Keratinisation (arrow) and keratin pearls (arrowhead), as well as (**B**) intercellular bridges (arrows) enable the histomorphological typing of a non-small-cell carcinoma as a squamous cell carcinoma. **C,** Another region of the same carcinoma, with a highly infiltrative growth pattern with tumour buds (arrowheads) and perineural invasion (arrow). **D,** Squamous cell carcinoma *in situ* in the main bronchus, extending into the epithelium of the bronchial ducts (arrow and inset).

subdivided into keratinising, non-keratinising and basaloid SCC.[1] It can be diagnosed readily without immunohistochemistry in morphologically unambiguous, i.e. keratinising, tumours (Figure 1). Nonetheless, the threshold to perform confirmatory immunohistochemistry should be low, in particular if tumours are non-keratinising (Figure 2), as these might finally represent, for example, adenocarcinomas or small-cell carcinomas, which comprise the most frequent differential diagnoses in the conventional and basaloid morphological setting, respectively.[8] Keratinisation needs to be convincing and must be distinguished from apoptosis and necrosis. Occasional cells with eosinophilic/orangeophilic cytoplasm may represent apoptotic or necrotic cells and need to be distinguished from true keratinisation. It is important to note that the presence of intracellular mucin in some scattered tumour cells does not exclude SCC. According to the WHO classification, more than five intracytoplasmic mucin droplets in at least two high-power fields point to adenocarcinoma or an adenocarcinoma component of an adenosquamous carcinoma.

SCC usually has a solid growth pattern, but may feature a pseudo-glandular/cribriform or alveolar filling growth pattern or may line the alveolar walls growing below the pneumocytic layer, imitating lepidic adenocarcinoma. Rarely, SCC may present with a focally clear cell phenotype. SCC may also display spindle cell morphology, but if spindle cells comprise > 10% of the tumour, it falls into the diagnostic category of pleomorphic carcinoma.[1]

In the subtype of basaloid SCC, the basaloid component should comprise at least 50% of the whole tumour.[1] Basaloid SCC is characterised by small- to intermediate-sized cells with scant cytoplasm and nucleolated nuclei, growing in nests with peripheral palisading (Figure 3). Although they usually lack squamous morphology, keratin pearls may be seen,

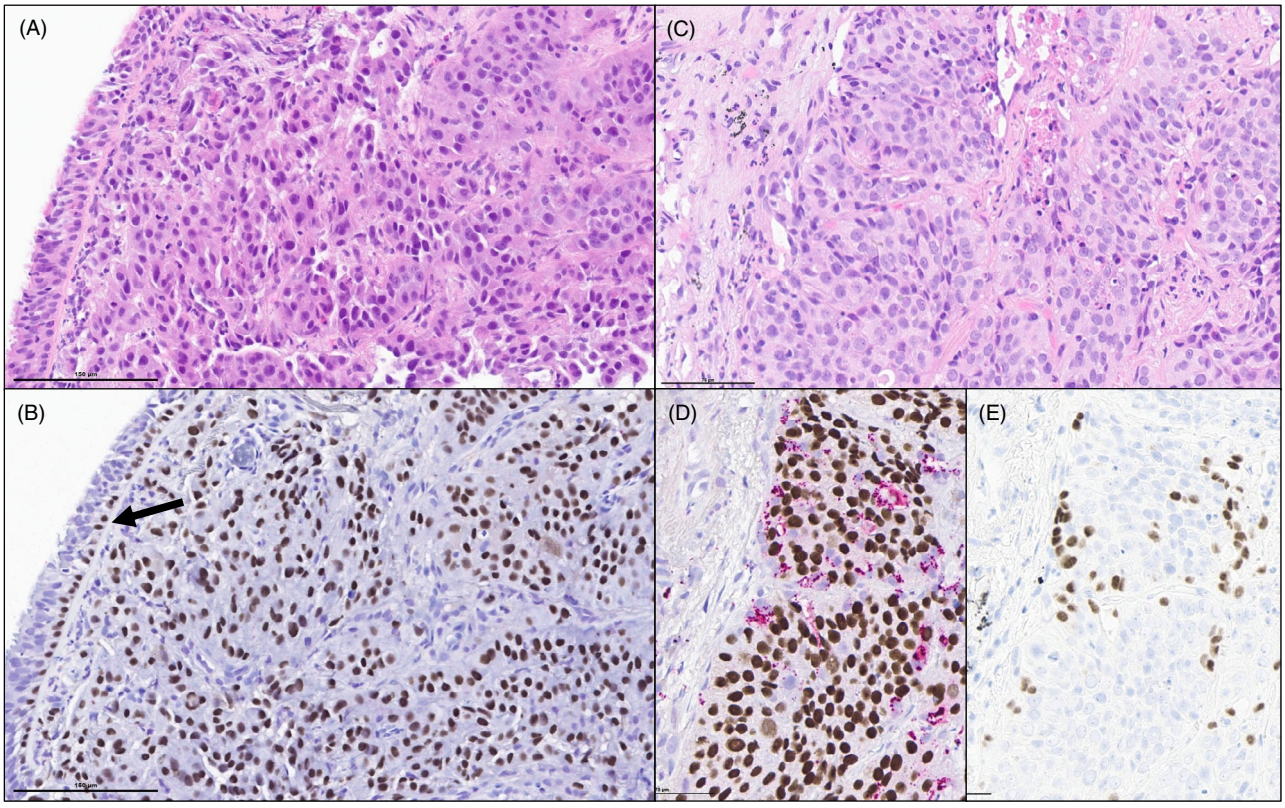

Figure 2. Non-keratinising squamous cell carcinoma. **A,** Non-small-cell carcinoma featuring a solid growth pattern and lacking keratinisation or intercellular bridges. **B,** More than 50% of the tumour cells express p40, without any TTF1 expression (not shown), enabling the diagnosis of 'non-small-cell carcinoma, favour squamous cell carcinoma' in this bronchial biopsy. Note the bronchial epithelium with p40-positive basal cells (arrow), representing an internal positive control. **C,D,** This non-keratinising squamous cell carcinoma shows an equally strong nuclear p40 expression in the tumour cells. Napsin A (red, granular staining) depicts the residual/entrapped pneumocytes at the periphery of the tumour nests. **E,** The pneumocytes are highlighted with TTF1 and should not be confused with an adenocarcinoma component of the tumour.

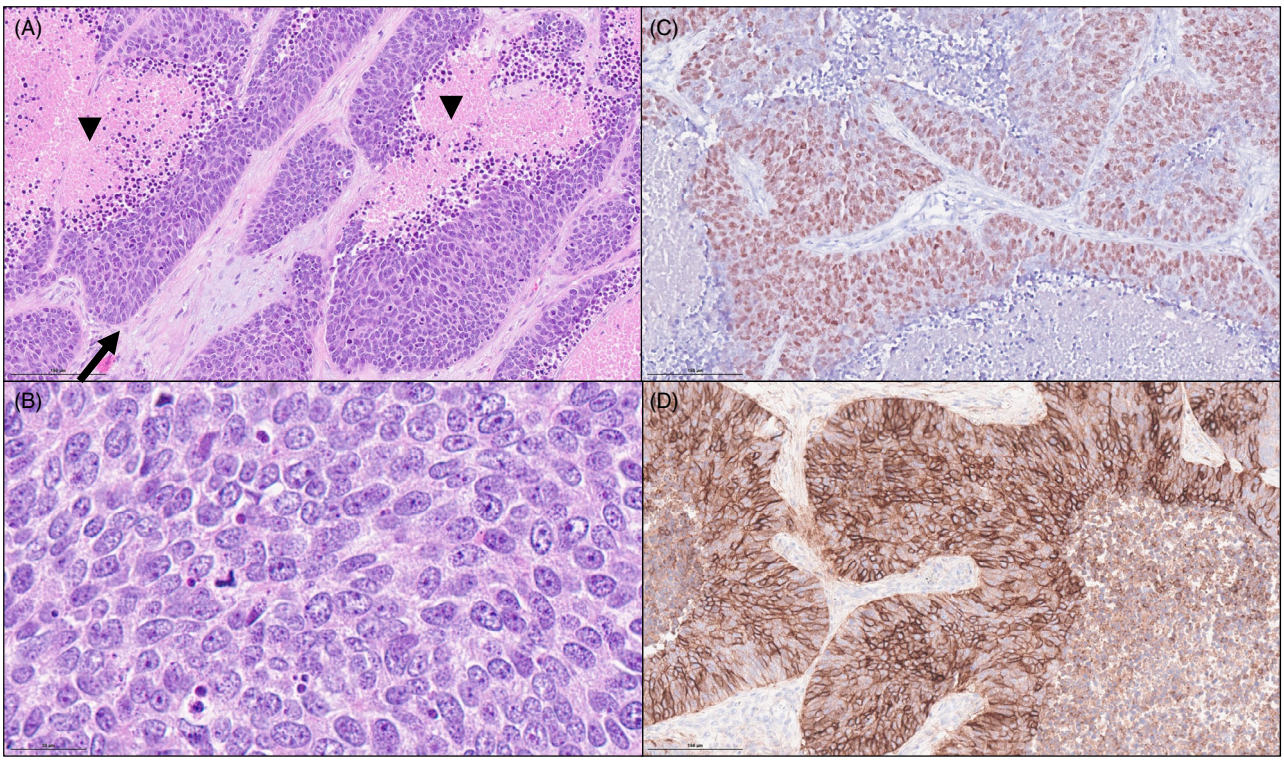

Figure 3. Basaloid squamous cell carcinoma. **A,** The tumour nests show peripheral palisading (arrow) and extensive necrosis (arrowheads). **B,** The tumour cells are of intermediate size with nucleoli and scant cytoplasm (H&E). **C,** Expression of p40 in > 50% of tumour cells establishes squamous differentiation in this non-keratinising carcinoma. **D,** Note that CD56 may be diffusely expressed, as in this case, without alluding to neuroendocrine differentiation.

and they reliably express p40. The proliferation index assessed by Ki67/MIB1 should exceed 50%.

Immunohistochemistry

Immunohistochemistry confirmatory for SCC comprises TTF1 negativity and positivity of p40 in the majority of tumour cells (at least 50%);[9,10] p40 is preferred over p63 as a squamous marker due to its higher specificity. Since the 2015 WHO classification, resected tumours are diagnosed as SCC, despite lacking squamous morphology if they display a squamous immunophenotype.[11] In small biopsies and cytology specimens, the diagnosis of 'non-small-cell carcinoma, favour squamous cell carcinoma' is rendered in morphologically nondescript carcinomas with convincing p40-positivity (Figure 2).[1]

Expression of the high-molecular-weight cytokeratins CK5/6 or 34βE12 is not sufficient for typing as SCC in morphologically ambiguous tumours, but helps to characterise the tumour as NSCLC and differentiates it from small-cell lung carcinoma. Similarly, expression of CK7 cannot be used for excluding the SCC nature of a carcinoma, as it is positive in a significant number of SCCs (44% of 202 SCC; 21% of 456 SCC; own unpublished data: 28/331 SCC, 8.5%).[12,13]

Focal and scattered positivity (< 5%) for p40 may be present in adenocarcinoma and does not prove squamous co-differentiation. It was reported in up to 16% of adenocarcinomas of the lung.[14,15] In contrast, there are rare cases of tumours with true bilineage differentiation and strong TTF1 and p40 double-positivity in the individual tumour cells, most often displaying a morphology of non-keratinising SCC with possible keratin pearl formation[16] (Figure 4). A recent publication, featuring the largest cohort of those cancers to date ($n = 14$), proposes the new diagnostic term of 'NSCLC with biphenotypic differentiation' for this rare group of carcinomas not yet specifically included in the WHO classification.[1,16] These should not be diagnosed as conventional SCC, as in some cases they feature differing molecular characteristics with targetable mutations (*EGFR*, *KRAS* G12C), nor should they be diagnosed as adenocarcinomas, as they frequently harbour *FGFR1*

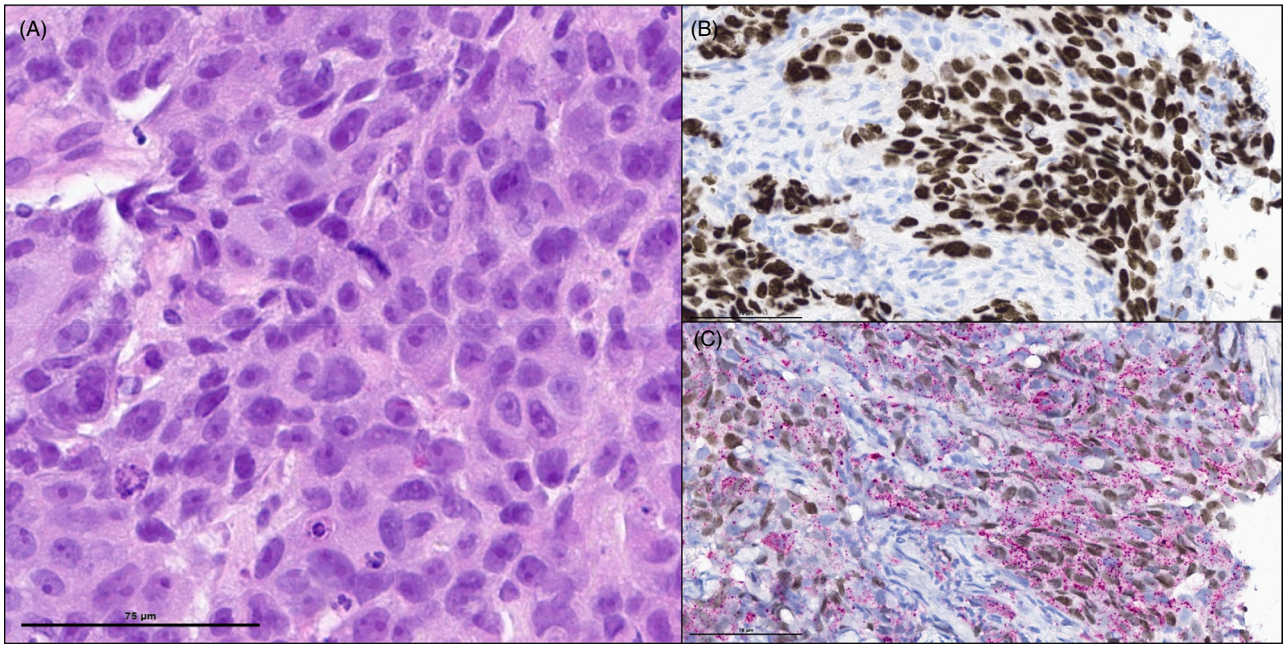

Figure 4. Non-small-cell carcinoma with biphenotypical differentiation, showing the solid growth pattern of a non-keratinising carcinoma (CA), with co-expression of TTF1 (B), Napsin A and p40 (C) in the individual tumour cells.

amplifications.[16] It is thus advisable to always test for both TTF1 and p40 in morphologically ambiguous cases. Of note, the first thoracic *DEK*::*AFF2* fusion carcinoma described also co-expressed TTF1 and p40 (see Differential diagnosis section).[17]

Caution is advised in using neuroendocrine markers, which may be positive in conventional SCC and do not speak *per se* in favour of a neuroendocrine carcinoma. In particular, the non-specific positivity for CD56 is a well-known phenomenon and is particularly common in SCC with basaloid features.[18]

Grading

There is no established and universally acknowledged grading system for SCC of the lung. In other neoplasms, grading is usually at least partially based on the degree of differentiation, proliferation and architectural patterns. This is the case for adenocarcinoma of the lung and neuroendocrine tumours, and it has been shown to have prognostic significance.[1,19] Until the 2015 edition of the WHO classification for pulmonary neoplasms, SCC was classified in line with the extent of keratinisation as well, moderately and poorly differentiated, but those categories failed to predict its behaviour.[20] The prognostic value of the three current categories (keratinising, non-keratinising and basaloid) is still imperfect.[21–23] Thus, additional histological criteria are currently evaluated for inclusion into a grading system; some have shown promising prognostic value, such as tumour budding (Figure 1C) and nuclear diameter.[22–25] The prognostic impact of the presence of 'spread through air spaces' (STAS) has also been reported by some groups, but not by others.[26–28] A project by the pathology committee of the International Association of the Study of Lung Cancer (IASLC) to propose a grading system for SCC is currently under way.

SCC *in situ*

Squamous cell carcinoma *in situ* (SCIS) is the precursor to SCC, is often multifocal and similarly strongly associated with cigarette smoking (Figure 1D). Preinvasive lesions are usually asymptomatic and diagnosed during bronchoscopy. SCIS typically arises within the central airways near the bifurcations in the segmental bronchi, and may precede or accompany invasive SCC.[29,30]

SCIS forms part of a continuum of morphological changes within the bronchial epithelium. The normal human airway epithelium is comprised of four major cell types, including ciliated, secretory, intermediate and basal cells. With chronic smoking, alterations within the bronchial epithelium occur, beginning with basal cell hyperplasia, followed by loss of ciliated

cells, squamous metaplasia and loss of cell junctions.[31–33] Dysplasia, SCIS and invasive SCC may develop. Key histological features used in the grading of dysplasia and diagnosis of SCIS include epithelial thickness, orientation and maturation of the basal cells, cell shape and size, nuclear changes and mitotic activity.[34] The main features distinguishing SCIS from high-grade dysplasia are the absence of maturation and loss of polarity of the nuclei in relation to the basement membrane.

Although generally thought to be a sequential progression from one end to the other, this is not necessarily the case, with some pre-invasive lesions remaining stable or even regressing upon repeat biopsy.[30,35] During long-term evaluation of mild and moderate dysplasia, regression was observed in 64%, stabilisation in 22% and progression in 14% of cases.[30,36] Progressive lesions have been shown to share several genomic, transcriptomic and epigenetic changes with invasive SCC, including marked chromosomal instability.[37] By contrast, regressive lesions have fewer chromosomal gains and losses, and gene expression and DNA methylation profiles closer to normal bronchial epithelium.[37] It is acknowledged that squamous cell dysplasia is not an easy diagnosis, as extensive cytological atypia may also be present in reactive and metaplastic lesions. On a personal note, we have never diagnosed mild dysplasia to date in our case load, as we feel that there is too much overlap with reactive lesions.

Advances in image-enhanced bronchoscopy, including high-resolution fluorescence bronchoscopy methods, have led to an increased detection rate of centrally located premalignant lesions. These lesions have a characteristic fluorescence pattern which may present macroscopically and/or histologically as a polypoid or a flat lesion, with an irregularly thickened and highly vascular mucosal surface.[38] This correlates with the variable morphology of dysplastic proliferations, which may be papillary and/or angiogenic.[38,39]

Cytology in the diagnosis of SCC

ACQUISITION OF MATERIAL

Cytology allows for a reliable diagnosis of SCC, and the evaluation of (mediastinal) lymph node involvement by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a cornerstone of lung cancer diagnosis and staging.[40] Bronchial washings, bronchial brushings, collection of sputum, bronchioalveolar lavage (BAL) and thoracentesis of pleural effusion are additional cytology-sample acquisition techniques. Rapid on-site examination (ROSE) can inform the interventionist during the procedure if the material collected is of adequate quality and quantity for diagnosis, avoiding the necessity of a repeat biopsy, and may also provide a preliminary diagnosis.[41–44]

Cytological material does not suffer from fixation and cutting artefacts and allows clearer appreciation of nuclear and cytoplasmic details. This advantage may also be exploited during intra-operative evaluation of lung nodules, where frozen sections can be complemented by smears or imprints (touch preparation) in order to evaluate cytological detail without freezing artefacts.

ANCILLARY TECHNIQUES

Immunohistochemistry can be performed on cytological material.[45–48] If the quantity of the sample allows, it is advised to generate a cytoblock of fixed cytological material for further immunohistochemical evaluation, but smears or cytospins may also be used.[49,50] Liquid-based cytology is less suitable, as methanol-based fixatives (such as CytoLyt) can produce background staining because of haemoglobin dissolution and reduce the intensity of immunohistochemical staining. Both DNA and RNA-based molecular analyses are possible on multiple preparations: direct smears, cytospins, cell blocks and cell-free liquid biopsy/supernatant from centrifuged specimens. Alcohol-fixed or air-dried specimens are both suitable for nucleic acid-based techniques such as next-generation sequencing (NGS).[51,52]

WHO CATEGORIES FOR REPORTING OF LUNG CYTOLOGY

In the recently developed WHO guidelines, five categories are recommended for reporting lung cytology: 'insufficient/inadequate/non-diagnostic', 'benign', 'atypical', 'suspicious for malignancy' and 'malignant'.[53] Regarding the first category, each laboratory should choose one terminology and consistently apply it if the material is quantitatively or qualitatively insufficient. The 'benign' category comprises inflammatory and infectious processes and benign neoplasms. The diagnosis should be as specific as possible. 'Atypical' comprises features that are predominantly seen in benign lesions and may indicate a malignant tumour, but are insufficient in quantity or quality to make a precise diagnosis. One challenge is that all types of cells in the lung can demonstrate atypia, including metaplastic squamous cells, reactive bronchial cells and histiocytes. 'Suspicious

for malignancy' with specification of the tumour suspected is rendered when there are some features suggestive of malignancy but insufficient in either number or quality to render an unequivocal diagnosis. The 'malignant' category, stating the specific tumour type, consists of cases with unequivocal findings.

CYTOMORPHOLOGICAL CRITERIA OF SCC

There are several cytomorphological characteristics of SCC on Papanicolaou (PAP)-stained smears.[54] The most specific feature is keratinisation, which can be obvious like keratin pearls or more subtle, such as dense orangeophilic cytoplasm. Intercellular bridges, typically visualised on histology, are only rarely identified. The material is usually highly cellular, with some single cells, small cohesive clusters or flat sheets of cells with a dense and sharply defined cytoplasm. The nuclei are usually centrally located, and either large, with angulated contours and irregular clumped chromatin, or small and hyperchromatic. Nucleoli are inconspicuous, except in poorly differentiated or non-keratinising SCC. Bizarre cells are often present, which may be very elongated ('fibre-cells') or tadpole-like. SCC is often accompanied by a 'dirty' background of necrotic debris and numerous neutrophils (Figure 5A,B).

SCIS or metaplastic cells can be sampled in sputum and bronchial brushings and washings and are difficult to distinguish from invasive SCC due to overlapping cytomorphological features. Atypical metaplastic and dysplastic cells are round to oval with well-defined borders, variable nuclear to cytoplasm (N/C) ratios and hyperchromatic, irregular nuclei. There are no definitive criteria to exclude an invasive neoplasm, but high cellularity and a dirty background with cellular debris point towards an invasive process.[55] The same applies to transbronchial lymph node fine-needle aspiration. If atypia is moderate, necrosis is absent and squamous cells are present in

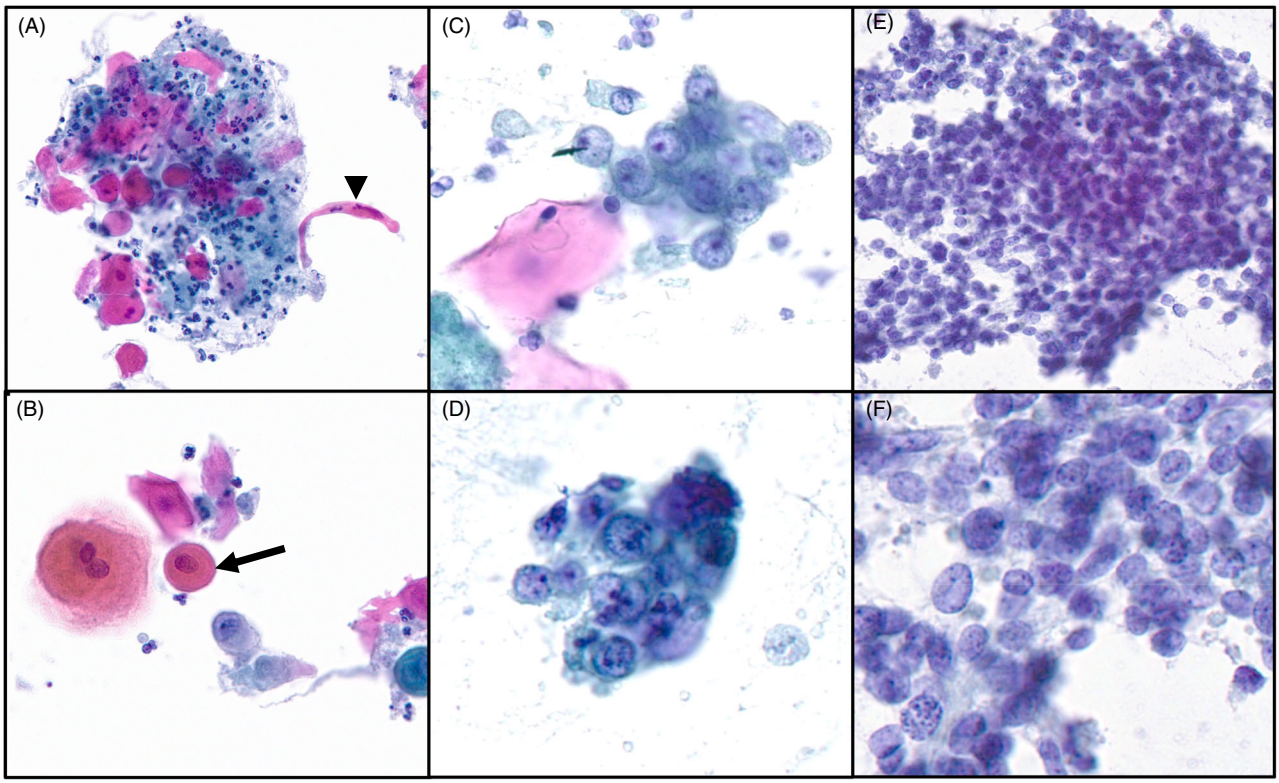

Figure 5. Cytological smears of the different subtypes of squamous cell carcinoma. **A,B,** Keratinising squamous cell carcinoma in a lymph node, acquired by EBUS-TBNA. Cells of various shapes, including 'fibre' cells (arrowhead), with some exhibiting typical dense orangeophilic cytoplasm (arrow). Note the dirty background with neutrophils and cellular debris. **C,D,** Non-keratinising squamous cell carcinoma in a bronchial aspirate. Three-dimensional clusters of undifferentiated cells, with moderately abundant cytoplasm and prominent nucleoli. Without immunostains, the diagnosis would be non-small cell carcinoma, NOS. **E,F,** Basaloid squamous cell carcinoma in a lymph node, acquired by EBUS-TBNA. Large sheets of small, uniform cells with practically no visible cytoplasm and finely granular chromatin, which closely resemble small cell carcinoma.

low numbers, a comment discussing the possibility of having sampled overlying dysplastic bronchial mucosa is advised. Other mimickers of malignant neoplasms include reactive changes due to inflammation, post-treatment or infections.[56,57]

Approximately 50–60% of tumours, which are finally diagnosed as poorly differentiated or, less frequently, basaloid SCC, lack sufficient morphological clues for squamous differentiation, i.e. keratinisation.[58] Non-keratinising carcinomas often form flat sheets or three-dimensional clusters with a variable number of isolated tumour cells, which often have a dense and well-defined cytoplasm. Nuclei are usually centrally placed, rounded or elongated and pleomorphic, with irregular chromatin and sometimes prominent nucleoli (Figure 5C,D). The basaloid SCC phenotype is difficult to identify on cytological preparations, but usually shows predominantly cohesive sheets of tissue fragments with occasional palisading and rarely pseudorosettes.[59,60] The N/C ratio is high, with a thin rim of cytoplasm, and round-to-oval nuclei with dense chromatin, usually without nucleoli. Nuclear moulding and crushing can occur, closely resembling small cell carcinoma. Immunostaining is mandatory to confirm the diagnosis (Figure 5E,F). The cytological diagnosis would be poorly differentiated SCC or SCC with basaloid morphology, as the quantitative definition of basaloid SCC cannot be evaluated.

Molecular alterations and their clinical significance

Chronic inhalation injury, most commonly in the form of tobacco smoking, causes prolonged exposure of bronchial epithelial cells to mutagenic and carcinogenic factors. The consequent DNA damage translates into a 'tobacco-smoke signature', characterised by an enrichment in GG > TT mutations, which is observed in pulmonary SCC as well as in other smoking-induced cancers, including SCC of the head and neck.[61]

Large-scale multiomic approaches have provided valuable insight into the molecular pathogenesis of pulmonary SCC.[62,63] Originally, four subtypes were described based on mRNA expression profiling, designated as primitive, classical, secretory and basal expression subtypes.[64] With increased available molecular data, including DNA methylation patterns and proteogenomics, additional molecular subtyping models have been proposed, highlighting the complex interplay of diverse tumorigenic mechanisms and the immune microenvironment, and providing potential prognostic information.[65–67]

Key molecular features unravelled by these studies involve a globally high level of genomic and chromosomal instability, which encompasses several recurrent alterations that may variably coexist and concur with the pathogenesis of invasive lung SCC. From a functional viewpoint, these alterations can be grouped according to the cellular process or signalling pathway that they affect: (i) PI3K and RAS pathways may be activated by mutations in *PIK3CA* or *PTEN* or amplification of *FGFR1* or *PDGFRA*; (ii) cell-cycle dysregulation occurs as a consequence of loss-of-function alterations in *CDKN2A*, *TP53* or *RB1*; (iii) response to oxidative stress is impaired by mutations in *NFE2L2*, *KEAP1*, *CUL3*; (iv) squamous differentiation is promoted by *SOX2* and *TP63* amplification or *NOTCH1* mutations; and (v) epigenetic regulation is altered by mutations in *KMT2D* or amplification of *NSD3*.[68–72]

Among the alterations mentioned, the following may be highlighted because of their high frequency in lung SCC: the tumour suppressor gene *CDKN2A*, which codes for the p16INK4A and p14ARF cell-cycle inhibitors, is inactivated in 72% of cases by mutations, promoter methylation or 9p deletions.[62] Moreover, *SOX2* and *TP63*, both encoding transcription factors that are critical for squamous cell differentiation, are located on the long arm of chromosome 3, in the region between 3q26 and 3q28, which is amplified in 25–75% of pulmonary SCC.[62,73,74]

Although some of these recurrent events may represent potential treatment targets, their value as actionable biomarkers in pulmonary SCC are still under investigation or have so far revealed themselves as unsatisfactory. Examples include *FGFR1* amplification (observed in 20% of lung SCC) and *PIK3CA* mutations/amplifications (in 5–15%/20% of the cases): clinical trials evaluating FGFR or PIK3CA inhibitors, respectively, in biomarker-selected patients have not yielded the expected benefits.[71,75] *SOX2* amplification/overexpression could be a more promising predictive biomarker: although SOX2 itself is not targetable, its chromatin regulators LSD1 and EZH2 can be inactivated by specific inhibitors that are being assessed in ongoing clinical trials.[71,76]

Conversely, several mutations and fusion genes that represent approved drug targets in pulmonary adenocarcinoma may also be detected in SCC, although at lower frequencies. Table 1 compares the prevalence of targetable oncogenic drivers in both NSCLC entities, including mutations in *EGFR*, *KRAS* (in particular the G12C mutation), *MET* (exon 14 skipping mutations), *ERBB2*, *BRAF* (V600E mutation) and fusion genes involving *ALK*, *ROS1*, *RET* or

NTRK.[77–101] Based on the probability of finding a therapeutic target, comprehensive molecular testing for these predictive biomarkers is recommended for all non-squamous NSCLC (when considering a systemic therapy) as well as for SCC in selected situations, in which a slight enrichment in targetable oncogenic drivers is to be expected: patients aged below 50 years, never smokers, former light smokers or long-term ex-smokers (who have quit smoking for more than 15 years).[84] This applies particularly to biopsies and cytological specimens where a diagnosis of SCC may actually represent an under-sampled adenosquamous carcinoma, with frequencies of oncogenic drivers more similar to adenocarcinoma.[82] Of note, in one study investigating 337 consecutive *MET* dysregulated stage IIIB/IV NSCLC, 6.8% of NSCLC with *MET* exon 14 skipping mutations were shown to be SCC, without age or histological particularities, favouring molecular testing regardless of histological subtype.[102]

Nonetheless, even in the presence of the same actionable *EGFR* mutations, it has been shown that patients with SCC have an inferior outcome following matched treatments compared to patients with adenocarcinoma.[103] Although the mechanisms underlying this relative intrinsic resistance are not fully understood, it is of particular relevance that squamous transformation is also a rare but well-recognised mechanism of acquired resistance in oncogene-addicted adenocarcinoma treated with tyrosine kinase inhibitors.[104] This phenomenon has been reported in both *EGFR*-mutated and *ALK*-rearranged lung adenocarcinomas following treatment with the corresponding inhibitors, and evidence has been provided that this type of histological transformation involves alterations in the PI3K/AKT signalling pathway.[105–107]

BASALOID SCC

Due to the lower frequency of basaloid SCCs, the molecular profile of these tumours has not been extensively studied. Most copy-number alterations have been shown to be similar between conventional SCC and basaloid SCC, while the latter are characterised by a distinct mRNA expression profile that involves up-regulation of embryonic stem cell markers and down-regulation of the squamous differentiation pathway. These observations are in line with the poorly differentiated status and aggressiveness of these tumours.[108] More recently, histopathological and molecular features in common with small-cell lung carcinoma (SCLC) have been evidenced. Interestingly, inhibition of the NOTCH pathway was found in more than 80% of basaloid SCC, in contrast to the aberrant activation of NOTCH signalling typically occurring in conventional lung SCC.[109,110] Decreased NOTCH pathway signalling, via down-regulation of NOTCH2 or up-regulation of ASCL1, may contribute to neuroendocrine differentiation in basaloid SCC, highlighting the similarity to the ASCL1-dominant SCLC subtype.[109,111,112] In addition, POU class 2 homeobox 3 (POU2F3), found in the tuft cell-like variant of SCLC with reduced expression of neuroendocrine markers, was reported in 22% of basaloid SCCs.[113]

Differential diagnosis

Differential diagnoses are summarised in Table 2 and comprise metastases, other tumours with squamous differentiation and tumours with morphological overlap but differing immunophenotype, which is a broad category in the non-keratinising SCC subtype. In addition, care needs to be taken not to interpret florid squamous metaplasia as SCC, as it may sometimes be extensive. This is frequently present in the vicinity of infarcts or associated with inflammation and underlying lung damage. Conversely, peripheral SCC may be necrotic with central cavitation featuring fungal or bacterial superinfections.

As there are no specific morphological or immunohistochemical characteristics for pulmonary, compared to extrapulmonary SCC, the main ways of differentiating a primary cancer from a SCC metastasis of another origin in routine daily practice are the clinical setting and radiological presentation. In those cases in which an *in-situ* SCC component is recognised and retrospective colonisation of the bronchial epithelium can be excluded, a primary pulmonary SCC can be assumed. Metastases from p16-positive SCC may be distinguished using p16-positivity as a first screening procedure. Subsequently, HPV-association needs to be validated using an alternative method such as HPV *in-situ* hybridisation, as non-HPV-associated tumours including lung carcinomas may be aberrantly p16-positive.[114,115] Although HPV may play a role in very rare and specific cases of SCC, as in the context of HIV infection and papillomatosis, no HPV association was detected in well-controlled studies in conventional SCC.[114–119] Also, the presence of PAX8 and CD5 and CD117 (c-Kit) co-expression speak in favour of a thymic origin of the SCC.[120]

Molecular analysis may also be utilised, with direct comparison between the extrapulmonary tumour and the pulmonary nodule. Of note, artificial neural

Table 1. Prevalence of targetable oncogenic drivers in pulmonary squamous cell carcinoma versus adenocarcinoma

Molecular alteration	Prevalence in pulmonary squamous cell carcinoma	Prevalence in pulmonary adenocarcinoma	Targeted treatment options*	References
EGFR mutations and indels				
EGFR exon 19 deletions and L858R mutation	3–7%**	10–15% in Western populations Up to 50% in Asian populations	EGFR tyrosine kinase inhibitors (TKIs) (e.g. osimertinib)	77–80
EGFR exon 20 insertions	< 1%	1–3%	Amivantamab (bispecific antibody targeting EGFR and MET) or mobocertinib (EGFR TKI)	
Other *EGFR* mutations (including G719X, S768I, L861Q)	< 1%	1–3%	Afatinib (second-generation EGFR TKI)	
KRAS mutations				
KRAS G12C mutation	1–3%**	10–15% in Western populations < 5% in Asian populations	Selective covalent KRAS G12C inhibitors (e.g. sotorasib, adagrasib)	77,83–88
Other *KRAS* mutations	1–3%**	8–20%	(under investigation: selective KRAS G12D inhibitors)	
MET exon 14 skipping mutations	1–5%**	3–4%	MET TKIs (e.g. capmatinib, tepotinib)	77,78,83,84,89,90
BRAF V600E mutation	< 1%	2–4%	Combination of dabrafenib (BRAF inhibitor) and trametinib (MEK1/2 inhibitor)	77,78,83,84,91,92
ERBB2 (HER2) mutations	1%**	1–3% in Western populations Up to 7% in Asian populations	Trastuzumab–deruxtecan (antibody–drug conjugate)	77,83,84,93–95
ALK fusions	1%**	4–7%	ALK TKIs (e.g. alectinib, brigatinib, lorlatinib)	77,83,84,96
ROS1 fusions	< 10 cases reported	1–3%	ROS1 TKIs (e.g. crizotinib)	77,83,84,97,98
RET fusions	1 case reported	1–2%	RET TKIs (e.g. selpercatinib)	77,83,84,99
NTRK fusions	0.1–0.5%	0.1–0.6%	TRK inhibitors (e.g. entrectinib, larotrectinib)	77,83,84,100,101
All potentially targetable alterations	~10%**	~50% in Western populations ~70% in Asian populations		

*The drugs indicated as examples are among those being EMA- and/or FDA-approved in NSCLC harbouring the corresponding drivers.
**May be over-represented, due to under-sampled adenosquamous carcinoma.

network machine-learning analysis of DNA methylation profiles was successfully applied to distinguish primary lung SCC from head and neck tumour metastases, but this has not yet been validated further for clinical use.[121] Regarding the distinction between metastases from a single pulmonary SCC and multiple independent pulmonary SCC, a recent study on multiple resected SCC nodules in the lung found that

Table 2. Differential diagnoses of pulmonary squamous cell carcinoma and lymphoepithelial carcinoma

	Differential diagnoses	Helpful findings in SCC/LEC distinguishing it from the DDx
Squamous cell carcinoma	Limited sampling of adenosquamous carcinoma	No adenocarcinoma component (beware of reactive pneumocytes adjacent to the tumour)
	Mucoepidermoid carcinoma	Keratinisation; no TTF1-negative glandular component; no *MAML2* translocation
	SMARCA4-deficient undifferentiated tumour	SMARCA4-IHC retained
	Thymic SCC	IHC profile (no PAX8 expression; no CD5 and CD117 co-expression); no thymic/mediastinal mass
	Metastasis from SCC	HPV-associated (male and female genital tract, head and neck): HPV-ISH* Not HPV-associated: no established markers to date, methylation analysis and certain IHC in research use
	Metastasis from urothelial carcinoma	IHC profile; clinical presentation
	Poorly differentiated (solid) adenocarcinoma	P40-positive and TTF1-negative; foci of squamous differentiation
Basaloid SCC	Small-cell lung carcinoma	p40-positive; strong and circular keratin-expression without dot-like positivity typical in SCLC; chromatin pattern
	Large-cell neuroendocrine carcinoma	p40-positive; chromatin pattern (beware – basaloid SCC may show some neuroendocrine marker expression)
	Lymphoma	IHC profile (keratin positivity)
	NUT carcinoma	NUT IHC-negative
	DEK::AFF2 carcinoma	No *DEK::AFF2* fusion
Lymphoepithelial carcinoma	Conventional SCC	Cytology and histomorphology; lymphoid infiltrate; EBER-ISH positivity
	Metastasis from nasopharyngeal carcinoma	Histologically undistinguishable; no nasopharyngeal mass on clinical presentation
	Thymoma/thymic carcinoma	IHC profile (no PAX8 expression; no CD5 and CD117 co-expression); no thymic/mediastinal mass
	Non-Hodgkin lymphoma	IHC profile (keratin positivity)

IHC, immunohistochemistry; ISH, *in-situ* hybridisation; LEC, lymphoepithelial carcinoma; SCC, squamous cell carcinoma.
*The use of p16 IHC is generally discouraged, as it may be positive in non-HPV-associated lung carcinomas; but if the status of the primary carcinoma (head and neck or cervical) is known, it may be used for screening.

histomorphological comparison fails to reproducibly distinguish between the two when compared to extensive molecular profiling.[122]

Metastases from carcinomas of other origins can sometimes mimic non-keratinising SCC, such as urothelial carcinoma. A panel including CK7, CK20, GATA-3, CK14, desmoglein-3 and uroplakin III may assist in distinguishing metastatic urothelial carcinoma from primary lung cancer. Expression of CK7 (100 versus 33%), CK20 (54 versus 7%) and GATA-3 (78 versus 23%) were reported to point towards urothelial carcinoma, whereas expression of CK14 (77 versus 32%) and desmoglein-3 (87 versus 11%) favoured SCC in a study comprising 30 and 38 cases per group.[123]

Mucoepidermoid carcinoma should be considered in non-keratinising, centrally located tumours in younger patients. It should show a mucin-producing glandular component negative for TTF1 and Napsin A. If detected, a *MAML2* rearrangement establishes the diagnosis.[124]

Basaloid SCC must be differentiated from its most important differential diagnosis of SCLC, which may be a mimicker on both histological and cytological preparations, warranting immunohistochemical confirmation. Unlike SCLC, tumour nuclei may be

nucleolated and lack the predominantly discohesive pattern and the finely granular chromatin ('salt and pepper'). Nuclear moulding and apoptotic debris are also generally more prominent in small-cell carcinoma. Basaloid SCC does not usually express the more specific neuroendocrine markers, but may be CD56-positive, highlighting the importance of p40 in this morphological group (Figure 3).

Poorly differentiated SCC can be mistaken morphologically for other tumours. Although well-differentiated adenocarcinoma typically has a columnar-like cell shape with vacuolated cytoplasm and eccentric vesicular nuclei, the morphology of the poorly differentiated counterpart may overlap with SCC, showing medium-sized cells with prominent nucleoli and dense cytoplasm (Figure 6). Due to this overlap, the addition of histochemical stains for mucin and a targeted immunohistochemical panel (p40, TTF1) is advocated.

Adenosquamous carcinoma is a differential diagnosis of SCC, particularly in small biopsies and limited sampling, where only the SCC component may have been captured. Adenosquamous carcinoma is composed of a mixture of variable proportions of adenocarcinoma and SCC components, with each accounting for at least 10% of the tumour. Importantly, the WHO essential diagnostic criteria require that these be two separate, distinct populations of cells.[1] When SCCs have morphologically distinct or poorly differentiated areas and foci of pseudoglandular differentiation, this can raise the possibility of an adenosquamous carcinoma. Mucin stains and/or immunohistochemistry (TTF1, p40) should be applied in this setting to verify the diagnosis.

NUT carcinomas are most often p40-positive and are a differential diagnosis for poorly differentiated keratinising and non-keratinising SCC and basaloid SCC. They exhibit a monomorphic morphology of small to intermediate-sized cells with prominent nucleoli and an associated neutrophilic infiltrate.[1,125] Abrupt keratinisation is characteristic, but seen in fewer than 50% of cases.[125] NUT carcinomas are highly proliferating and usually present in advanced stages. They are rare tumours, with a frequency of 0.6% reported in biopsies of lung carcinomas without glandular morphology.[126] The defining nuclear protein in testis (*NUTM1*)–gene rearrangement can be assessed by immunohistochemistry using NUT-specific antibodies, and the specific fusion may later be confirmed using sequencing methodology.

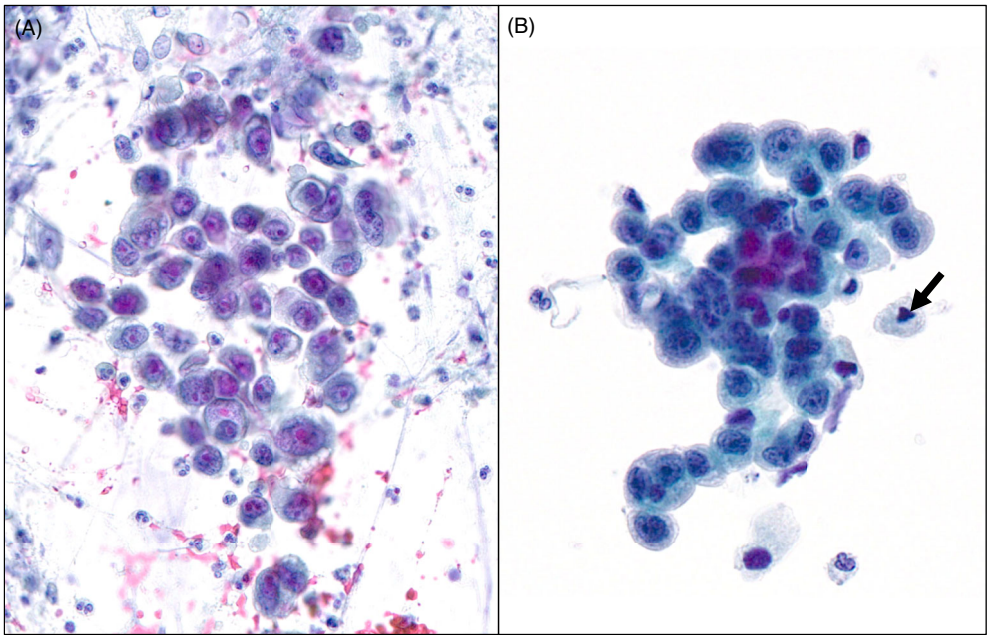

Figure 6. Cytological smears of poorly differentiated adenocarcinomas, which present a pitfall and differential diagnosis of squamous cell carcinoma. **A,** Bronchial aspirate (PAP stain) featuring large cells with well-defined cytoplasmic borders and irregular nuclei with prominent nucleoli. These morphological characteristics are nondescript and can also be seen in non-keratinising squamous cell carcinoma. **B,** This metastatic adenocarcinoma in a lymph node, sampled by EBUS-TBNA, shares some of the characteristics of squamous cell carcinoma; notably, dense cytoplasm and hyperchromatic nuclei, which can sometimes be pycnotic (arrow).

SMARCA4-deficient undifferentiated tumours are also smoking-related and, by definition, poorly differentiated carcinomas often with rhabdoid morphology, which can show focal p40 expression.[127] Immunohistochemistry for SMARCA4 will establish the diagnosis.

Another rare differential diagnosis for SCC and SCCs with basaloid features is *DEK::AFF2* carcinoma, defined by the *DEK* (exon 7)::*AFF2* (several breakpoints, exons 4, 5, 6, 9) fusion, which may be detected using a fusion-specific antibody.[128] *DEK::AFF2* carcinoma was initially described in the head and neck region, and recently as a central lung mass in a young never-smoking woman.[17,129] The histological hallmarks are a mixed exophytic and endophytic pattern of papillary intraluminal component and invasive anastomosing ribbons with basaloid features, lack of overt keratinisation (although focal squamous pearls may be seen) and cytological monotony.

Finally, metastatic melanoma should always be ruled out in keratin-negative lung nodules, as this great mimicker may present in various morphological flavours.

Lymphoepithelial carcinoma

The WHO essential diagnostic criteria of LEC are predominantly histomorphological and comprise a non-keratinising SCC histology with syncytial-appearing tumour cells with indistinct cytoplasmic borders. The nuclei are large and vesicular with distinct eosinophilic nucleoli, embedded in a usually prominent lymphoplasmacytic infiltrate, which lies between and within tumour islands[1] (Figure 7). Focal keratinisation, spindle cell growth and intratumoral amyloid deposition may occur.[130]

Approximately 95–100% of pulmonary lymphoepithelial carcinomas in Asian patients are associated with EBV,[131–133] whereas in Caucasian patients an association between EBV infection and pulmonary LEC is not typically observed.[134–136] Therefore, EBER *in-situ* hybridisation positivity is currently only a desirable, but not an essential diagnostic WHO

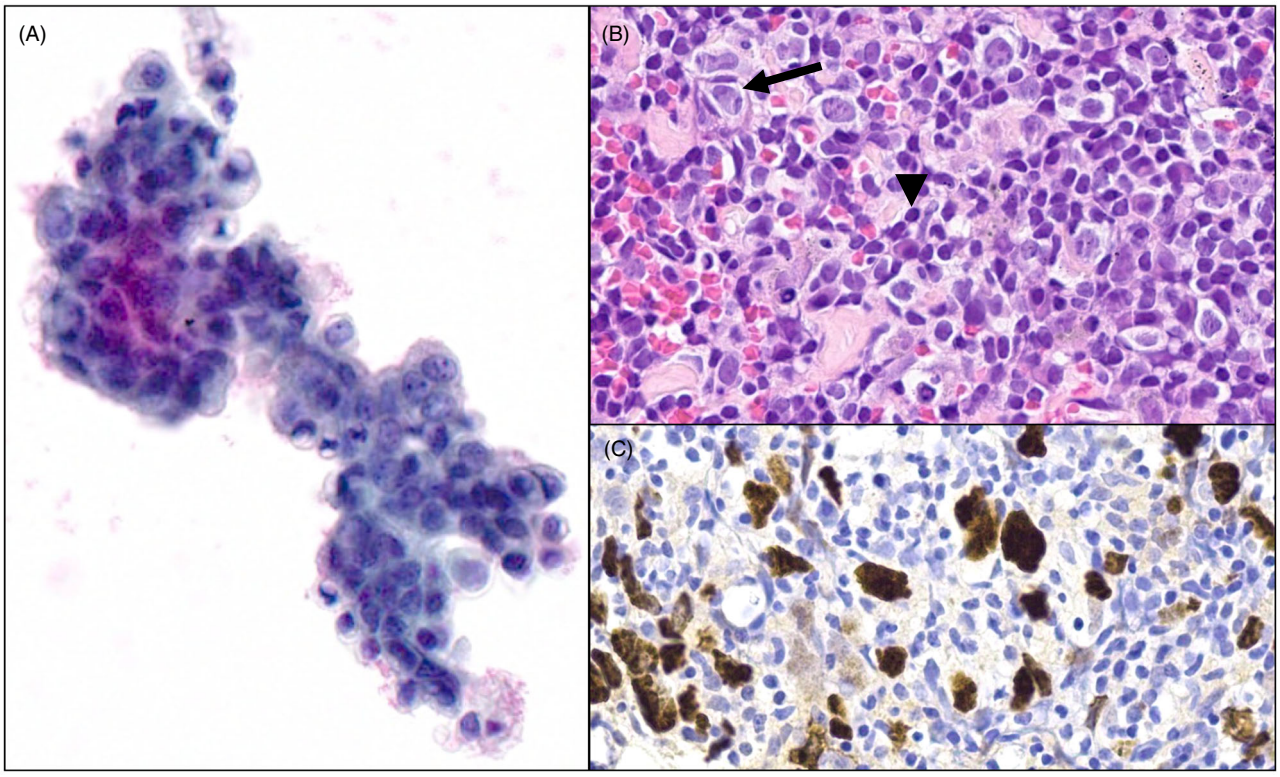

Figure 7. Metastatic lymphoepithelial carcinoma in a lymph node, sampled by EBUS-TBNA. **A,** A cluster of undifferentiated cells with well-defined borders, moderately abundant cytoplasm and coarse nuclear chromatin. **B,** In a cell block preparation the tumour presents as large, atypical cells (arrow), admixed with numerous small lymphocytes (arrowhead; H&E stain). **C,** EBER ISH confirms EBV association, highlighting the positive carcinoma cells (highlighted in brown).

criterion, rendering the diagnosis ambiguous and presumably obscuring a clearly outlined diagnostic entity of LEC. EBER *in-situ* hybridisation is positive in the epithelial tumour component and negative in the associated lymphoid tissue (Figure 7C). Cytomorphological characteristics of LEC of the lung are not well-described and non-specific. The cells generally form large clusters with brisk mitotic activity and contain large hyperchromatic nuclei, usually with prominent nucleoli, scant cytoplasm and an extensive interspersed lymphoid infiltrate.[137] Due to the indistinguishable histological and cytological presentation, it is paramount to clinically exclude a metastatic nasopharyngeal carcinoma (NPC).

Recently, the genomic landscape of EBV-associated LEC has been described, exhibiting a low mutational burden but widespread copy-number variations. Altered pathways include NF-κB, JAK/STAT and cell cycle. Overall, EBV-related LEC show similarities with NPC, while their molecular features are clearly distinct from other NSCLC.[133,138] The molecular pathogenesis of EBV-negative LEC remains largely unknown.

Treatment and prognosis

Complete resection of the tumour by anatomical resection and lymph node dissection is the treatment of choice in early stages of SCC and LEC, complemented by neoadjuvant and/or adjuvant chemotherapy, radiotherapy and/or immunotherapy in locally advanced tumours. All tumours should be evaluated for programmed death ligand 1 (PD-L1) expression, as this may guide therapy, e.g. identifying patients most probably responding to first-line anti PD-1/PD-L1 therapy.[139] The addition of immunotherapy to chemotherapy results in survival benefit in patients with advanced LEC.[140–142] LEC is documented to have better survival rates compared to conventional NSCLC in both early and advanced stages.

Conclusion

Pulmonary SCC is the second most common histological type of NSCLC. Extensive research during the last decade has helped to refine the diagnostic criteria of this tumour, especially the usage of immunohistochemistry to type morphologically ambiguous lesions and its molecular background. However, challenges remain on the diagnostic and treatment front. Current histomorphological challenges encompass a grading system and the possibility to separate primary lung tumours from SCC metastases using methodology applicable in daily routine. Lastly, personalisation of treatment has been lagging behind due to the lack of targetable molecular alterations in the majority of SCC. Currently, international guidelines recommend routine molecular testing only in non-squamous lung carcinomas, but this might change due to increasing awareness of the presence of targetable mutations also in SCC patients regardless of age or smoking history, although the clinical significance still needs to be elucidated.[87,103]

Ambiguous diagnostic criteria are challenging in the diagnosis of LEC, as not all LEC are reported to be associated with EBV and not all EBV-associated lung tumours feature a prominent lymphoid infiltrate. Overlapping and ambiguous cases will occur and warrant further sharpening of the essential diagnostic criteria in this rare tumour entity.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Data availability statement

Data sharing is not applicable to this article, as no new data were created or analysed in this study.

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