Histopathology

Histopathology 2022, **80**, 457–467. DOI: 10.1111/his.14537

REVIEW

Elastin in pulmonary pathology: relevance in tumours with a lepidic or papillary appearance. A comprehensive understanding from a morphological viewpoint

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(2022) *Histopathology* **80**, 457–467. https://doi.org/10.1111/his.14537

Elastin in pulmonary pathology: relevance in tumours with a lepidic or papillary appearance. A comprehensive understanding from a morphological viewpoint

Elastin and collagen are the main components of the lung connective tissue network, and together provide the lung with elasticity and tensile strength. In pulmonary pathology, elastin staining is used to variable extents in different countries. These uses include evaluation of the pleura in staging, and the distinction of invasion from collapse of alveoli after surgery (iatrogenic collapse). In the latter, elastin staining is used to highlight distorted but pre-existing alveolar architecture from true invasion. In addition to variable levels of use and experience, the interpretation of elastin staining in some adenocarcinomas leads to interpretative differences between collapsed lepidic patterns and true papillary patterns. This review aims to summarise the existing data on the use of elastin staining in pulmonary pathology, on the basis of literature data and morphological characteristics. The effect of iatrogenic collapse and the interpretation of elastin staining in pulmonary adenocarcinomas is discussed in detail, especially for the distinction between lepidic patterns and papillary carcinoma.

Keywords: diagnosis, elastin, lung, pathology, review

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Introduction

In pulmonary pathology, elastin staining is used to variable extents in different countries. In some

settings, it is used to distinguish collapsed alveoli from papillary adenocarcinoma. This practice is not uniformly accepted, and, as a result, this has not been incorporated into the World Health Organization (WHO) definitions of lepidic and papillary adenocarcinoma.

The theoretical basis for the use of elastin staining is that normal lung tissue and pleura have elastic tissue that helps to delineate the histology of these lung structures. As a result, pathological conditions involving the lung and pleura can alter this architecture, and this alteration can be exploited diagnostically. However, there are differences in opinion on the value of the presence or absence of elastin once normal lung tissue has been altered by disease. In pulmonary adenocarcinoma, some use the presence of elastin as an argument for pre-existing structure [implying the diagnosis of adenocarcinoma *in situ* (AIS) or a lepidic pattern as part of a minimally invasive adenocarcinoma or lepidic-predominant adenocarcinoma (LPA)] to highlight the retention of existing architecture. Others emphasise the lack of elastin as an argument for papillary carcinoma, whether by destruction of elastic tissue or the lack of it in a new proliferation; in either event, this pattern would indicate invasion. These considerations are critical, as a collapsed lepidic pattern would not be included in the T stage assessment of invasive size. To complicate matters further, the term 'collapse' is used in different ways in the literature.

This review aims to describe the morphological structure and function of elastin in normal and diseased lung, and apply this to unravel interpretation issues mentioned above in pulmonary adenocarcinomas.

Elastin components and structure

The human lung is an intricate organ whose architecture includes the vasculature, conducting airways, and terminal airspace compartments, which need an elastic and balanced extracellular matrix to support repeated movements of extension and recoil throughout life.[1] Elastin and collagen are the main components of the lung connective tissue network, and together provide the lung with elasticity and tensile strength.[2]

The extracellular matrix has been defined as the structural network of collagens, elastin, glycoproteins and proteoglycans surrounding stromal cells and underlying endothelial and epithelial cells. In addition to having structural properties, the extracellular matrix functions as a dynamic modulator of various biological processes.[1] This is accomplished through the selective binding and subsequent release of growth factors and cytokines, and through its interaction with cell surface receptors.[3,4] Although collagen and elastic fibres are the major constituents of the extracellular matrix, the overall function is defined by the interrelationships between all of the various components.

Tropoelastin is the soluble monomer precursor of elastin, and is secreted as a 60-kDa mature protein produced, through variable splicing, by diverse elastogenic cell types in the lung, including chondroblasts, myofibroblasts, mesothelial cells, and smooth muscle cells.[5] Tropoelastin self-aggregates on the cell surface before being deposited onto fibrillar microfibrils and crosslinked to form elastic fibres, in a complex multistep process collectively referred to as elastogenesis.[3,5–7] Tropoelastin has a unique structure, possessing a mosaic of domains in various states of order. The free energy landscape of tropoelastin encompasses multiple energy minima with no sizeable barriers between them.[5,8] The molecule transitions easily between these low energy minima, giving rise to a conformational ensemble that comprises a wide array of structurally related but dissimilar states. This allows flexibility at a molecular level, providing, at the end of elastogenesis, the functional elasticity required in lung parenchyma for breathing, i.e. maintaining the patency of alveoli, small airways, and adjacent lymph vessels.

Elastin expression occurs over a narrow window of development, beginning in mid-gestation and continuing at high levels throughout the postnatal period.[9] The mature elastic fibre is an insoluble and stable protein with a very long lifespan of 80 years,[10,11] consistent with the lack of tropoelastin expression in adults (animal model[9]). Furthermore, elastin is extensively distributed in most human lung compartments, including the pleura, alveolar septa, large vessels, and cartilage.[9] The crude connective tissue dry weight concentrations of elastin are 20–30% in the respiratory parenchyma, 7–16% in the pulmonary blood vessels, and 3–5% in the airways.

The elastic fibre scaffold is known to be an important supportive structure of the normal alveoli.[9] Alveolar size increases with age.[2] The delicate three-dimensional network forms a looping system encircling the alveoli and alveolar duct, and ensures that applied forces will be transmitted equally to all parts of the lung.[9]

In functional terms, during exhalation the elastic fibres recoil and maintain a regular, spaced alveolar structure and the diameter of the small airways. A histochemical elastic fibre stain may be useful for recognition of the underlying pulmonary architecture.

Two forms of elastic fibre have been described in ultrastructural and three-dimensional image studies: thick and thin elastic fibres. The main framework of the alveoli is constructed of thick elastic fibres, forming the alveolar orifice and also the sides of the polygonal alveoli where three neighbouring alveoli join. Thin elastic fibres branch from the thick elastic fibres intercrossed in the alveolar wall, and support the alveolar wall.[12–14] Type II pneumocytes are located along thick elastic fibres.[14]

In elastin-stained histological 3–5-μm sections, thick elastic fibres may easily be recognised, whereas thin elastic fibres cannot readily be discerned. The light-microscopic literature on elastin usually deals with thick elastic fibres, which will be subsequently referred to as 'elastic fibres'. In two-dimensional histological sections of normal alveolar walls, the elastic fibres appear discontinuous curvilinear or dot-like[2,15] (Figure 1A–C). It is of note that these seemingly fragmented elastic fibres form part of the three-dimensional elastin network (scaffold); that is, in serial sections they are connected to each other.

Pathophysiology of elastin

Alveolar myofibroblasts are located underneath the alveolar epithelium, and are defined by expression of α-smooth muscle actin and the production of elastin and collagen.[16] In alveolar development, deposition of elastin is an essential process for septation. Elastin allows alveoli to stretch during inhalation.[17] After birth, elastin forms a matrix, serving as a scaffold on which alveolar myofibroblasts adhere and mark the sites of secondary septa.[16] Cyclic mechanical stretch is important to maintain the alveolar myofibroblast state.[18] In a model system of pulmonary fibrosis, the mechanical stretch is reduced and myofibroblast differentiation is increased.[19] Moreover, pre-existing elastin has been shown to induce an increase in extracellular elastin production by myofibroblasts. This explains the focal increase in the amount of elastin in areas with pre-existing elastin. As well as the increased expression of elastin, expression levels of the α1 chain of type V collagen and tenascin C are increased.[19] In chronic obstructive pulmonary disease, abnormal fibulin-5 metabolism is suggested to play a role in disturbed elastogenesis.[20]

In pleuroparenchymal fibroelastosis, the combination of increased numbers of subepithelial myofibroblasts and increased amounts of elastin in continuous sheets was demonstrated in areas with mild non-specific interstitial pneumonia.[21] This distribution of elastic fibres is similar to that seen in some cases of AIS and lepidic adenocarcinoma. These findings suggest that: (i) in AIS and non-specific interstitial pneumonia, an interaction between epithelial cells, extracellular matrix components and subepithelial myofibroblasts leads to increased elastin production; and (ii) the light-microscopic demonstration of continuous elastic fibres (corresponding to 'elastin sheets' in the third dimension) cannot be used as a criterion for invasion.

In pulmonary adenocarcinomas, Matsubara et al.[22] emphasised two locations of myofibroblasts, i.e. the above-mentioned alveolar subepithelial myofibroblasts and the stromal myofibroblasts, often in areas of central fibroelastosis. The subepithelial myofibroblast pattern is associated with a favourable prognosis.[22]

Terminology of elastin degradation

In 2000, Fukushima et al. used the term 'degradation of elastin' in an electron-microscopic study for the examination of pulmonary carcinomas. On ultrastructural examination (high magnification), some thick elastic fibres showed vacuolar changes and electron-dense granular deposits.[23] These changes were called 'degradation' of elastic fibres. Thus, the term 'elastin degradation', in this sense, constitutes an electron-microscopic definition of individual fibres, referring to features that are not readily recognisable with light microscopy.

In pulmonary adenocarcinomas, Eto et al.[24] performed a light-microscopic histological image analysis study using elastin stains, and noticed, in the central fibrotic 'invasive' part of peripheral adenocarcinomas, a disrupted pattern of the elastotic framework related to invasion and a poor prognosis. It is of note that they described, in the periphery of the tumour, a thin-walled elastic framework similar to normal alveolar walls. Fukushima et al.[23] compared elastin in non-malignant peripheral lung with that in well-differentiated adenocarcinomas, and found a normal pattern or an increase in the amount of elastin, but not a decrease in the latter.

Overall, these finding indicate that normal lung and lepidic-pattern tumours either retained normal elastin or showed thick elastin, but that disruption/degradation of elastic fibres occurred in central fibrotic areas only, in association with invasion.

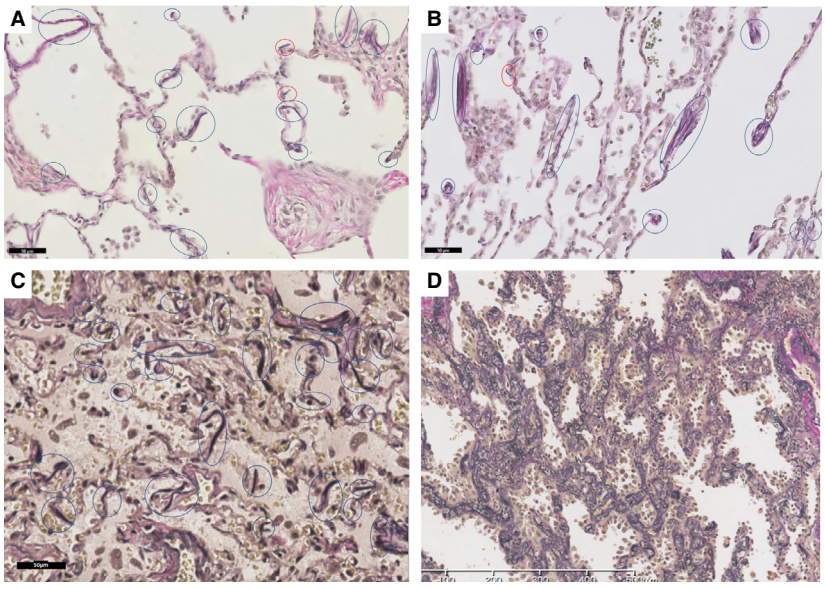

Figure 1. A,B, Two examples of elastin staining in a non-malignant peripheral lung with minimal iatrogenic collapse (perfusion-fixed). **A,** A 31-year-old male with organising pneumonia. **B,** A 39-year-old female with invasive mucinous adenocarcinoma. **C,** A specimen submerged in fixative with prominent iatrogenic collapse. The diagnosis was made on other sections of the same resection specimen. Circles (blue) encompass elastic fibres. Note: (i) the reduction in the amount of air because of collapse; (ii) that the number of discontinuous elastin fragments is dependent on the number of alveolar cross-sections in the field of view; (iii) that the alveolar cross-sections sometimes result in a pseudopapillary appearance; and (iv) that if one were to replace all pneumocyte type I cells lining the alveolar walls with a monolayer of cylindrical tumour cells, the proper diagnosis would be adenocarcinoma *in situ* (AIS), whereas, in terms of patterns, this may be perceived as a mixture of a lepidic pattern and a papillary pattern. The use of an elastin stain would reveal the underlying architecture of the pre-existing lung, as proven by the inherent distribution of fragmented elastic fibres, including some of the alveolar wall parts without elastin. **D,** Elastin staining of AIS showing the pre-existing alveolar structure with mild iatrogenic collapse (perfusion-fixed). Note the continuous sheets of elastin (compatible with Noguchi type B[27]).

Collapse

In the pulmonary pathology literature, two different descriptions have been used for 'collapse'. In 1985, the term 'collapse' was used in adenocarcinomas with a central 'scar', showing a recognisable collapsed alveolar framework within condensed elastic tissue on elastin staining. With haematoxylin and eosin staining, the condensed elastic tissue could be mistaken for fibrosis. In fact, elastin staining emphasised the preponderance of elastic over collagenous tissue.[25] This was also noted by Shimosato et al.[26,27] and supported by Yamashiro et al.[28]: the central part of the tumour shows collapsed alveolar spaces (absence of air) with condensation of elastin. However, these condensations of elastic fibres with some fibrosis are not infrequent. In this setting, fibrosis is defined as collagenous,[29] whereby elastosis is not an essential component of the fibrotic reaction. In the Japanese literature, the central part may be fibrotic as well as elastotic, whereas, in the above-mentioned 1985 study, this central area was characterized by elastin condensation without fibrosis. This meaning of collapse is a 'biological' collapse with loss of alveolar architecture. This alteration is present before the surgical procedure, and does not include the area outside the scar with peripheral lepidic growth and possible increased alveolar wall thickness.

Another definition of collapse[30] involves 'iatrogenic collapse'—i.e. an artefact of deflation—whereby the alveolar air, vascular blood and lymph volumes are reduced during surgery. Owing to the lack of negative pressure between the pleural leaves, the natural recoiling of the elastic fibres places alveolar walls in close proximity to each other.[30] This effect can be seen as an 'iatrogenic' or 'mechanical' 'collapse'; here, the alveolar architecture is maintained but is harder to evaluate morphologically. Examples of collapse involving areas with lepidic growth have recently been published.[31] The effect of iatrogenic collapse can be reduced during gross handling, e.g. by bronchial and/or transpleural perfusion with formalin. The distance between the alveolar walls will be enlarged by the perfusion process, but the effect of the iatrogenic collapse may not be fully mitigated. The enlarged formalin-containing spaces between the

alveolar walls are, light-microscopically, the equivalent of air-filled (clear) spaces. Gross handling differs between laboratories throughout the world: Japanese laboratories[14,24,32] and some laboratories outside Japan perform perfusion fixation,[33] whereas many pathology laboratories in the world will have the influence of prominently collapsed lung tissue in the diagnostic process. Perfusion fixation of the human lung has been performed for research[34–38] and clinically[39] in the past. For optimal preservation of the alveolar architecture, a perfusion fixation method may be chosen.[32,33] For an understanding of the published images of adenocarcinoma, it is useful if these studies mention the routine handling procedure for pulmonary resection specimens (including wedge resections). Overall, in contrast to biological collapse involving a central area of the tumour, the structural change of iatrogenic collapse is most prominent in light-microscopic evaluation at the level of the alveolar walls.

In some cases of AIS and LPA, the elastic fibres are increased in number and lie in sheets,[9] which, light-microscopically, are characterised by continuous elastic fibres. Noguchi type B is a clear example.[27] This increase in the number of elastic fibres emphasises the pre-existing lepidic (alveolar) structure (Figure 1D). Eto et al.[24] demonstrated centrally, in what is now called AIS, an increase in the elastin content, contraction of the alveolar wall, and a consequent marked reduction in the amount of remaining alveolar air. This phenomenon of possible *in-vivo* collapse of peripheral lung tissue was recently given support in a radiological–pathological correlation, whereby the radiological solid appearance could only be explained by collapse of the lepidic parts of adenocarcinomas.[31] A gross picture of the resection specimen associated with *in-vivo* collapse is shown in Figure S1. Thus, collapse of the peripheral lung will not only occur *ex vivo* during surgery, but may also happen *in vivo*.

Pathology of elastin

The diseases in pulmonary pathology with a change in elastin configuration are summarised in Table 1.

Table 1. Diseases in pulmonary pathology with a change in elastin configuration

Elastin	Disease	Morphology	Reference
Loss	Emphysema	Irregular enlarged airspaces and reduction in peribronchiolar alveolar wall attachments	Wright et al.[48] Kawabata et al.[49]
	Langerhans cell histiocytosis	Focal emphysematous change	Fukuda et al.[50]
	Granulomatous inflammation	Initial reticulin fibre increase in and around granuloma, resulting in hyalinosis	Mariani et al.[51]
	Idiopathic interstitial pneumonias	Fibrotic areas: focal elastolysis	Honda et al.[52]
Increase	Adenocarcinoma*	Increased elastin in alveolar walls and/or the collapsed centre	Masayuki et al.[27]
	Elastosis†	Dense disorganised deposits of elastin	Fukushima et al.[23] Starcher et al.[53]
	Apical cap	Subpleural increase in alveolar walls	Lagstein[54]
	Pleuropulmonary fibroelastosis	Subpleural and septal increase	Tsubosaka et al.[21] von der Thüsen et al.[55] Kinoshita et al.[56]
	Idiopathic interstitial pneumonias	Increased vascular elastin	Parra et al.[57]
	Organised infarct	Collapsed entangled elastic fibres within a collagenous stroma	Kawabata et al.[58] Groshong et al.[59]

*Some, but not all, adenocarcinomas have increased amounts of elastin in alveolar walls and/or the collapsed centre.
†Elastosis is defined as 'large aggregates of elastin fibres'/'diffuse increase in elastin mass', similarly as in other organs: breast,[60] stomach,[60] and papillary thyroid carcinoma.[60] The prognosis of patients with adenocarcinomas in the breast[61,62] and lung[23] is better in patients with elastosis than in those without elastosis.

In pulmonary adenocarcinoma, elastin staining can be used to demonstrate invasion in several ways. The most obvious are pleural and vascular invasion. Also, in pre-existing disease the elastin pattern can be helpful. The elastin pattern in seemingly papillary structures, which could be either true papillary or pseudopapillary because of iatrogenic collapse, requires more explanation.

PLEURAL INVASION

Recognition of the normal pleural elastin layer is used for staging. In small adenocarcinomas, elastin staining plays a role in determining the presence or absence of pleural invasion, defined as cancer cells infiltrating beyond the outer elastic layer or beyond the outer elastic layer and onto the visceral pleural surface.[40] Tumours ≤30 mm with pleural invasion are upstaged to pT2a (Figure 2).

VASCULAR INVASION

If a pathologist is in doubt about whether a histological structure is an artery or a vein, elastin staining may help in the recognition of vascular structure and thereby invasion by tumour cells: the elastic fibres in the tunica media of the pulmonary arteries will designate the vascular lumen.

PRE-EXISTING DISEASE

Adenocarcinoma may arise as a secondary disease on top of other pre-existing diseases, such as emphysema, so there may be an already modified tissue architecture. When the pattern of remodelling in the adenocarcinoma component is similar to that in the adjacent non-malignant lung with pre-existing disease, remodelling is probably due to the pre-existing disease and not to adenocarcinoma.

ADENOCARCINOMA, INCLUDING MIMICKERS OF PAPILLARY ADENOCARCINOMA

Iatrogenic collapse in a lung with non-mucinous AIS may mimic a papillary and/or acinar pattern as defined according to the WHO classification.[41] In this differential diagnosis, the presence of elastin shows that these structures represent pre-existing collapsed alveolar walls, and should be interpreted as part of the pre-existing peripheral pulmonary framework,[42] supporting the diagnosis of AIS or, if invasion is present elsewhere, a lepidic pattern of adenocarcinoma.

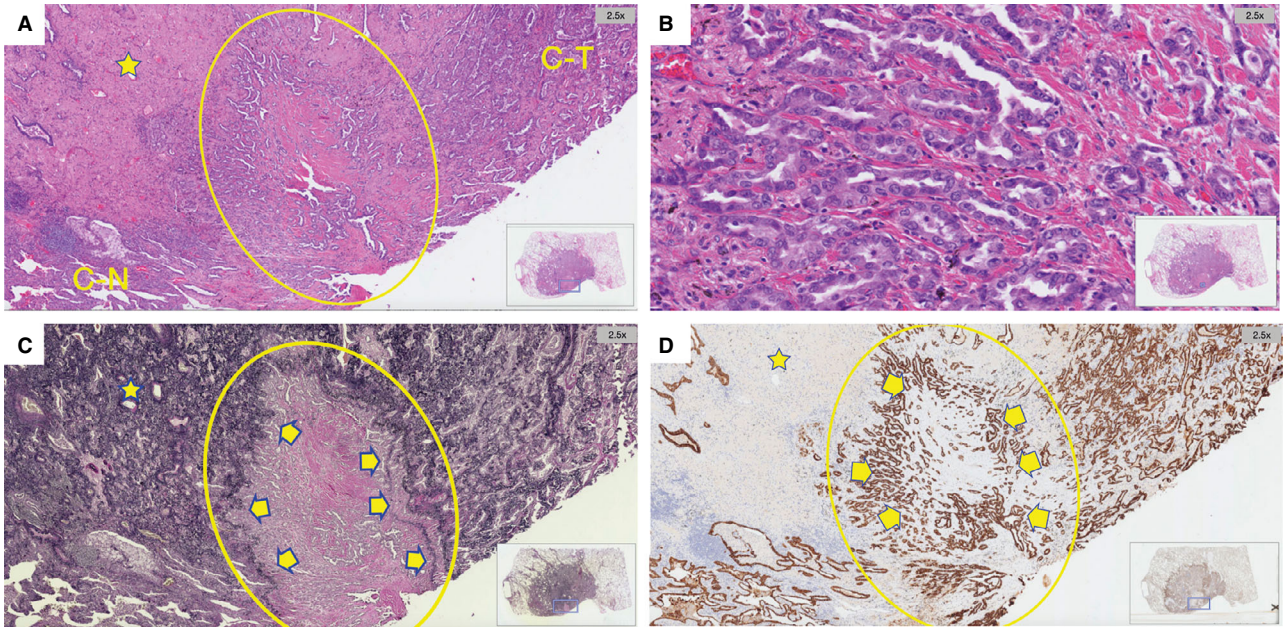

Figure 2. **A,B,** Overview (**A**) and detail (**B**) of the pleural invasive area: haematoxylin and eosin staining of the submerged fixed specimen shows invasive adenocarcinoma with pleural retraction (yellow oval) and a fibroelastotic scar (*yellow star). **C,** Corresponding elastin staining of A, showing elastin (black) in the lung as well as fibrosis (red) in the pleural retraction invaded by small acinar adenocarcinoma beyond the outer elastic layer (yellow arrowheads). **D,** Corresponding cytokeratin 7 staining to A, highlights cytokeratin 7-positive cells (brown). C-N, collapsed non-malignant area; C-T, collapsed tumour area.

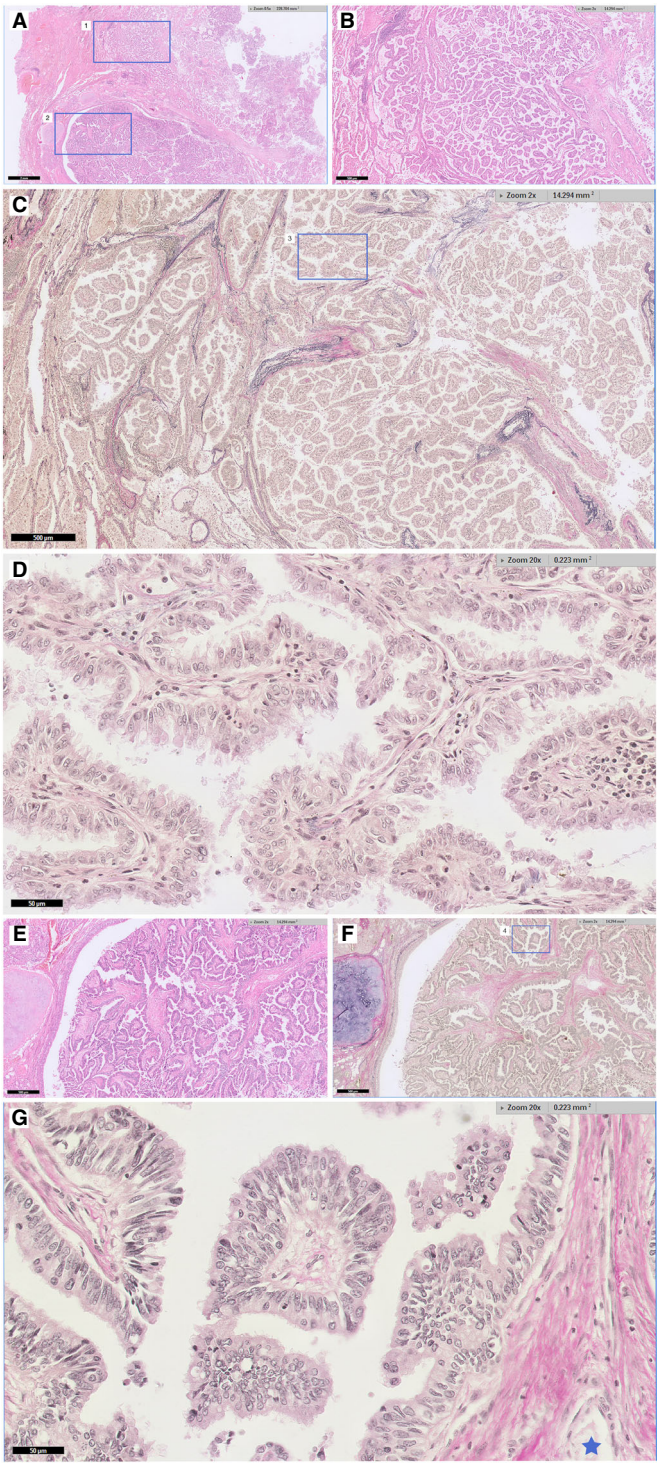

Figure 3. A submerged fixed resection specimen with papillary carcinoma. **A,** A haematoxylin and eosin (H&E) overview with both peripheral (around rectangle 1) and intrabronchial (around rectangle 2) growth. Note the iatrogenic collapse in peripheral non-malignant lung tissue in the left of rectangle 1. **B,C,** H&E (**B**) and elastin (**C**) staining show details of rectangle 1. Note the presence of elastin-containing remnants of the pre-existing architecture (including vessels and alveolar walls). Rectangle 3 is depicted in (**C**). **D,** Higher magnification of elastin staining of rectangle 3. Note the absence of elastin in all of the small papillae. **E,F,** H&E (**E**) and elastin (**F**) staining show details of rectangle 2. The internal control of the elastin staining (around the cartilage; below the bronchial epithelium and vessels) is positive. Rectangle 4 is depicted in (**F**). **G,** Higher magnification of elastin staining of rectangle 4. Note the complete absence of elastin intrabronchially, in the small papillae as well as in a slightly larger vessel (lumen with asterisk). [Colour figure can be viewed at wileyonlinelibrary.com]

At the centre of the controversy is the fact that true papillary cores include endothelial and fibroblastic cells with extracellular matrix, but elastic fibres are not structural components in papillary adenocarcinoma, unless there is pre-existing elastosis (Table 1). An example of a true papillary carcinoma of the lung is shown in Figure 3. The papillary carcinoma with partly intrabronchial location was chosen, so that it could not be confused with alveolar collapse.

AIS is influenced by iatrogenic collapse, and has a simple pattern with an epithelial monolayer and, frequently, thin alveolar stromal cores (Figure 4). The non-invasive nature of this papillary mimicker was recognised in 2005 by Ishikawa et al. as 'Noninvasive papillary adenocarcinoma of the lung: a proposal of new entity'.[43] These pseudopapillary structures result from iatrogenic collapse but should not be considered as a distinct pathological entity. Instead, this entity represents a gap in the definition of the 1999 WHO classification,[44] in which specific architectural and stromal constituents, such as desmoplasia, were not incorporated.[45]

Importantly, cross-sections of these papillary mimickers have a diameter of 46 μm (range, 36–54 μm; Motoi and Thunnissen, unpublished data). The microscopic two-dimensional view of a papilla is approximately representing the middle of the three-dimensional papillary structure. One would expect that cutting deeper sections from the paraffin block would lead to disappearance of the papillary structure in four to five serial sections (Figure S2).

The morphological criteria in the above-mentioned differential diagnosis, i.e. (i) the presence of elastin in alveolar walls, (ii) the absence of elastin in true papillary carcinoma,[42] and (iii) the three-dimensional size of these papillary mimickers, point to AIS or a lepidic pattern.

The application of the current WHO classification was discussed among the authors. A case with iatrogenic collapse with tumour cells in one or two layers along a thin wall led to agreement. However, when elastin was absent in similar structures, opinions ranged from a lepidic pattern to possible papillary outgrowth (and therefore invasion), see Figure S3.

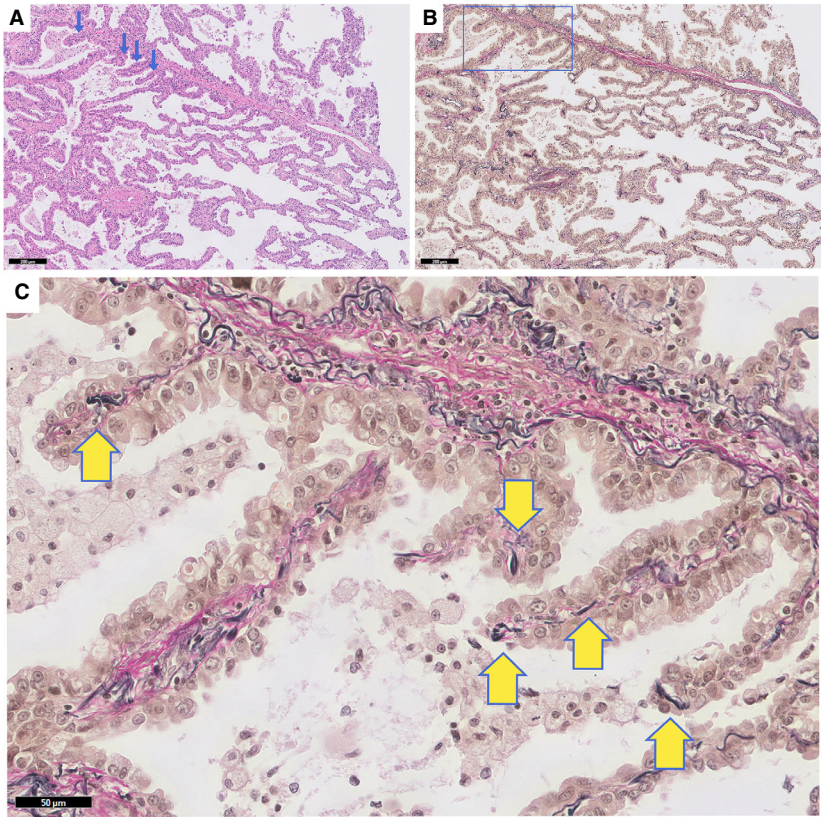

Figure 4. **A,B,** An example of lepidic adenocarcinoma with a pseudopapillary pattern (**A,** haematoxylin and eosin; **B,** elastin) with a focal papillary mimicker (blue arrows). **C,** Elastin staining shows the same pseudopapillae with focal elastin (yellow arrows). The stromal part of papillary carcinoma is mainly collagenous and focally desmoplastic. Note that the elastin in the interlobular septum is curly, as opposed to being stretched during maximal inhalation. [Colour figure can be viewed at wileyonlinelibrary.com]

The difference in interpretation may result from arguments based on a retained background architecture (i.e. the collapsed underlying microscopic anatomy), whereas the criteria used in the WHO classification of lung cancer since 1999 are based on microscopically visual tumour cell patterns. Although it is realised that the iatrogenic collapse may have an effect on measurements of tumour size,[40] the WHO does not incorporate in the current lung cancer classification the effect of iatrogenic collapse on pulmonary adenocarcinomas.[30] It is clear that more data on this subject with sufficient follow-up are needed to resolve this uncertainty, in order to reach the level required for an evidence-based pathology classification.

DESMOPLASTIC STROMA

A mixture of tumour cells and dot-like or fragmented elastic fibres in combination with an increase in the number of loose collagen fibres (desmoplastic stroma[46]; neofibrogenesis) is characteristic of stromal invasion. Usually, these tumour cells have high-grade atypia. Tumour areas with desmoplastic stroma and/or other classic characteristics of invasion do not form part of the above discussion regarding a collapsed AIS/lepidic pattern and papillary adenocarcinoma.

ARTEFACT

A seemingly focal increase in elastin content occurs in cases of pulmonary collapse after surgery as an *ex-vivo* artefact: the bronchiolar epithelial layer folds because of the smooth muscle contraction. Underneath this folded epithelial layer is an accumulation of recoiled elastin.[30] Remarkably, the simple epithelium lining *in vivo* is flexible enough to pile up as small folds in in the bronchiolar lumens of lungs with iatrogenic collapse. It is not excluded that, in collapsed AIS, some piling of the tumour cells on alveolar walls may occur. If this does not consistently exceed a thickness of two cell layers, early data support an excellent prognosis.[47]

Conclusions

Overall, iatrogenic collapse may have a profound effect on the microscopic appearance in pulmonary adenocarcinomas. Perfusion fixation, as routinely used in some centres, may mitigate this effect. Elastin staining is useful for the distinction between AIS with iatrogenic collapse and papillary carcinoma. Use of elastin staining should be considered, in order to make the most accurate diagnosis.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

E. Thunnissen designed the review. N. Motoi, Y. Minami, D. Matsubara, Y. Nakatani, Y. Ishikawa, T. Radonic, A. C, Borczuk, M. Noguchi, H. Blaauwgeers and E. Thunnissen contributed essentially to the neoplastic content and wrote the paper. W. Timens and X. Baez-Navarro contributed essentially to the elastin content and wrote the paper. All authors approved the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Example of tumor atelectasis*) In A and B sequential slices of a lobectomy specimen after 24+ hours buffered formaldehyde fixation. The orientation of 16 slices was as described before [33]. The fibrous adhesion on the left(upper) side was during gross examination colored with red ink. The slices were numbered from crania(a)l (1) to cauda(a)l (16) direction. The slice orientation is shown in the right upper corner (Media(a)l = m; Ventra(a)l = v; Dorsa(a)l = D; Latera(a)l = L). The letters D-G adjacent to the slices denote location of sample taken for initial microscopic analysis. Note i) that peripheral areas were fixed (grey) and some central areas are unfixed (red). In addition, ii) the slices do not show a solid lesion (which is associated with a firm consistency). iii) The bronchial and vascular resection margins as wells as lymph nodes were sampled before cutting the 16 slices. In C and D microscopic images of haematoxylin-eosin stained section (sampled from slice 12, location N) showing overview (C) and detail (D) with monolayer of tumor cells on the alveolar wall (compatible with AIS). Note i) the variability in the amount of 'air' space: more collapse is less 'air'; and ii) in these images there are no signs of invasion. *) Shown upon request of a few reviewers. Ambrosi F, Lissenberg-Witte B, Comans E, et al. Tumor atelectasis gives rise to a solid appearance in pulmonary adenocarcinomas on HR-CT. *JTO Clin. Res. Reports* 2020; **1**; 1-10.

Figure S1B

Figure S1C

Figure S1D

Figure S2. A: Cartoon with change of true papillary structure in consecutive series of sections. Note that a true papillary structure (with a radius of about 23 mm) should disappear after 4 – 6 sections. B: Cartoon with cross-section of alveolar wall. Although the shape may change, the cross section can be followed in serial sections.

Figure S3. A flow chart with thought process for the differential diagnosis between collapsed AIS versus papillary/ other adenocarcinomas. *) In serial sections of collapsed AIS continuity of the alveolar walls is visible, with the exception of cross sections of alveolar duct endings. "Of note, in desmoplastic stroma elastic fibers may also appear discontinuous due to degradation of elastin by elastase containing inflammatory cells (see text for location of elastin degradation). Moreover, the desmoplastic stroma is a morphologic clue towards invasion."