Chapter 2
Mechanisms of Lung Cyst Formation

Debbie Clements, Roya Babaei-Jadidi, and Simon R. Johnson

Introduction

Lung cysts are air-filled spaces with clearly defined walls of less than 2 mm surrounded by lung parenchyma. Cystic lung diseases are characterised by tens to many thousands of intrapulmonary cysts which may range in size from millimeters to centimeters. Whilst cysts are usually described as round, such as in lymphangioleiomyomatosis (LAM), in other conditions, including pulmonary Langerhans cell histiocytosis (PLCH), cysts are irregularly shaped and arise from airways rather than in the parenchyma. In this chapter, we shall describe the current thinking around mechanisms of cyst formation in four representative diseases, LAM, Birt-Hogg-Dubé syndrome (BHD), PLCH and protein deposition-related cystic lung disease. Whilst the initiating mechanism of pathologic destruction in LAM, BHD and PLCH is a consequence of dysregulation of specific molecular pathways driven by monogenic mutations, the mechanisms of parenchymal destruction in complex diseases including COPD have been more extensively studied and may help inform our understanding of mechanisms of parenchymal destruction in rare cystic lung diseases.

The lungs are constantly exposed to injurious inhaled stimuli throughout life. Loss of lung architecture by abnormally activated extracellular matrix-degrading proteases is considered to contribute to lung parenchymal destruction in a number of diseases including alpha 1 antitrypsin deficiency (A1ATD) and COPD. Whereas unregulated neutrophil elastase activity is the likely cause of degradation of elastin containing alveolar septae in A1ATD, the relationship between protease expression,

D. Clements · R. Babaei-Jadidi (✉)
Division of Respiratory Medicine, The University of Nottingham, Nottingham, UK
e-mail: debbie.clements@nottingham.ac.uk; roya.babei-jadidi@nottingham.ac.uk

S. R. Johnson
Division of Respiratory Medicine and Biomedical Research Centre, The University of Nottingham, Nottingham, UK
e-mail: simon.johnson@nottingham.ac.uk

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protease activation and parenchymal destruction in other lung diseases is less clear [1]. Further, inhibition of proteases in lung diseases, including metalloproteinase inhibition in COPD and doxycycline in LAM, has been largely unsuccessful as a treatment modality. This is likely due to the complexity of protease substrate profiles and interactions in vivo. For example, despite assignment to the family of the matrix-degrading proteases, the substrate profile of the macrophage-derived protease matrix metalloproteinase-12 (MMP-12) implicated in COPD includes a greater number of innate immune proteins, coagulation factors and bioactive molecules in addition to extracellular matrix (ECM) substrates [2]. A proteomic analysis of protease substrates in the airways of patients with COPD shows that during exacerbations, proteases cleave hundreds of non-ECM substrates, including a range of protease inhibitors, resulting in alterations in multiple protease classes and cleavage of multiple substrates [3]. Thus, inhibiting a single protease may have a large number of unpredictable and off-target effects.

Activation of proteases, reactive oxygen species and inflammatory pathways in response to a constant, low-level exposure to inhaled toxins and particles has the potential to cause lung injury. In healthy individuals, the resulting airway and alveolar damage is countered by homeostatic and repair mechanisms. Lung parenchymal loss is likely to be the result of either a large insult exceeding the lung's repair capacity, loss of protective and repair mechanisms or disease-related imbalance of injurious stimuli and repair mechanisms. Airway and alveolar progenitor/stem cells normally have the capacity to repair low-level insults caused by inhaled toxins. Repair mechanisms require the replication of alveolar or airway stem cells, including alveolar type II cells, to replace damaged alveolar epithelial type I cells. Alveolar repair is associated with the activation of alveolar developmental programs associated with lung development, particularly including the Wnt and platelet derived growth factor receptor (PDGFR) alpha pathways [4]. The replicative capacity of all cells, including alveolar stem cells, is limited. Repeated cell division and the cumulative burden of DNA mutations can predispose these cells to malignant transformation. Senescence, a mechanism which limits the absolute number of divisions made by a cell, not only protects against malignancies but also limits the capacity of tissue repair. Much of the current thinking around alveolar destruction in diseases including COPD is focused around loss of repair mechanisms by the combined impact of cell senescence and aging limiting the lungs repair capacity [5]. Senescence may occur as a response to multiple stimuli, including multiple cell divisions (replicative senescence), inflammation, reactive oxygen species or genetic activation of specific molecular pathways including the mechanistic target of rapamycin (mTOR) pathway. When alveolar epithelial cell replacement programmes are functional, the presence of an intact ECM scaffold is required for alveolar cell differentiation, survival and hence normal tissue repair.

In parenchymal lung destruction, the balance between disease-related lung injury and the efficiency of repair mechanisms is likely to maintain a balance between health, over-exuberant repair leading to fibrosis and parenchymal destruction resulting in cystic lung disease. In complex diseases such as COPD, multiple damage and repair pathways interact with each other to cause parenchymal lung damage. In rare

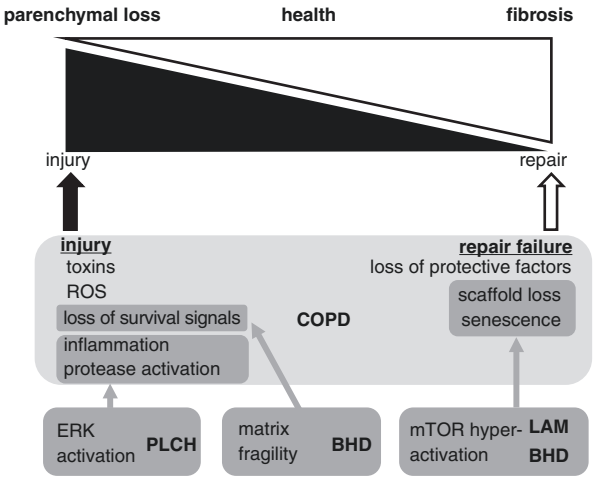

Fig. 2.1 Mechanisms of parenchymal damage in disease. Alveolar integrity in health is maintained by a balance between parenchymal injury and repair mechanisms. In diseases categorized by parenchymal destruction, including chronic obstructive pulmonary disease (COPD) or cystic lung diseases including pulmonary Langerhans cell histiocytosis (PLCH), Birt-Hogg-Dubé syndrome (BHD) or LAM, disease-related mechanisms result in parenchymal damage or impaired alveolar repair. In LAM, PLCH and BHD, specific molecular abnormalities result in primary derangement in specific processes, whereas polygenic/environmentally driven diseases such as COPD affect many injury and repair mechanisms simultaneously

diseases with a monogenic molecular etiology, disease mechanisms are likely to be more restricted to processes downstream of the molecular abnormality, such as mTOR activation in LAM and ERK activation in PLCH (Fig. 2.1).

Mechanisms of Cyst Formation in Specific Diseases

Lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a rare, multisystem disease that occurs predominantly in premenopausal women and involves the lungs and axial lymphatic system and is associated with the benign tumor angiomyolipoma [6]. It can occur sporadically (S-LAM) or in association with tuberous sclerosis complex (TSC-LAM).

The lungs and lymphatics of patients are infiltrated by smooth muscle-like ‘LAM cells’ leading to thin-walled pulmonary cysts and fluid-filled masses in the axial lymphatics [7]. Although LAM cells have a histologically benign phenotype, LAM is considered a low-grade malignant neoplasm by the World Health Organization (WHO). LAM cells can break away from the original lesion and spread (metastasize)

through the blood vessels or lymphatics [8] and can be detected in chylous fluid, blood and urine of patients [8]. The metastatic ability of the LAM cell was originally noted in patients with S-LAM, with identical *TSC2* mutations in the lung lesions and angiomyolipomas, and is also supported by the recurrence of LAM in the female recipient after transplantation from male donors [9]. Loss of lung function in patients with LAM is highly variable. The mean decline in forced expiratory volume in 1 second (FEV1) is 120–140 ml per year; some progress rapidly, whilst others can remain stable for many years [10].

LAM is often misdiagnosed and confused with other respiratory conditions [11]. Pulmonary function tests (PFTs) usually show an obstructive defect which can be mistaken for COPD or asthma. In 2010, the European Respiratory Society (ERS) laid down guidelines for the correct diagnosis of LAM and categorization of patients into three groups of definite LAM, probable LAM, and possible LAM based on high-resolution computed tomography scan (HRCT), clinical history and presentation, including a high level of the ymphangiogenic vascular endothelial vascular endothelial growth factor (VEGF-D) in the serum [12]. A recent study using unbiased serum proteomics identified that changes in vitamin D-binding protein (VTDB) and its gene, group-specific component (GC), are associated with LAM severity and survival [13].

Mechanistic target of rapamycin (mTOR) is a serine/threonine kinase member of the phosphoinositide 3-kinase (PI3K)-related kinase family. The protein is highly conserved from yeast to mammals [14]. mTOR is a component of two complexes: mTOR complex 1 (MTORC1) and mTOR complex 2 (MTORC2). mTOR complex 1 signalling regulates and promotes ribosomal biogenesis, protein synthesis, de novo lipid synthesis, nucleotide synthesis for DNA replication and proteasome-dependent proteolysis and suppresses catabolic pathways such as autophagy [15]. MTORC2 controls cell growth and survival by phosphorylating and activating PKA, PKG and other PKC protein kinases, including protein kinase B (AKT), which, when activated, facilitates proliferation, growth and cell survival [16].

Tuberous sclerosis complex (TSC) proteins 1 and 2 negatively control mTORC1. TSC is a heterotrimeric complex comprising TSC1 (hamartin), TSC2 (tuberin) and TBC1 domain family member 7 (TBC1D7) [17]. The protein complex is an upstream regulator of mTORC1, through GTPase-activating protein (GAP) for the Ras homolog enriched in the brain (Rheb) [18]. Mutations in the TSC1 and TSC2 genes result in the accumulation of active Rheb-GTP, stimulation of mTOR and phosphorylation of S6 kinase and eukaryotic initiation factor 4E-binding protein which lead to increased translation, cell size and proliferation. TSC-LAM is caused by mutations in either TSC1 or TSC2 genes [19]. In patients with TSC-LAM, a germline mutation is present in the TSC1 or TSC2 gene; the second mutation in the other TSC allele occurs in somatic tissue ('two-hit' mechanism) [20]. Although TSC-LAM occurs almost exclusively in women, it was also reported in 13%–38% of men with TSC [21, 22], albeit with milder severity than in women [22]. LAM also occurs in the absence of TSC germline mutation, in a sporadic form, caused by a somatic mutation in the TSC2 gene [23] with the second hit occurring after

conception in somatic tissues [24]. The pulmonary manifestations of S-LAM and TSC-LAM are nearly indistinguishable, although patients with TSC-LAM tend to be identified earlier, due to increased recognition of the disease or screening and as a consequence with better lung function in comparison to S-LAM [25]. S-LAM has only been reported in one male patient so far, and TSC gene mutations were not found in the lung tissue [26].

The origin of the LAM cell is unknown; these spindle-shaped cells usually can be found in small nodules in the lung in cyst walls and lymphatics. LAM cells express markers of both smooth muscle, including α-smooth muscle actin, vimentin and desmin, and melanocyte lineages such as gp100, MART-1, CD63 and PNL2 [27]. Due to the expression of melanocytic markers, it has been postulated that LAM cells originate from the neural crest [27]. LAM cells express estrogen and progesterone receptors [28] and belong to the perivascular epithelioid cell (PEC) group of neoplasms [29]. The LAM tumor is a complex structure containing TSC null ($TSC^{-/-}$) LAM cells and wild-type cells, including hyperplastic type II pneumocytes (positive for PE-10 or TTF-1 markers) lining the LAM nodules which appear to have apical microvilli and cytoplasmic projections [30]. Another cell type present in LAM lesions is the lymphatic endothelial cell, mostly located in intra-LAM lesion lymphatic channels [31]. It was once thought that the stromal cells within LAM nodules were primarily composed entirely of a single clone of LAM cells [32]; however, it is now believed that the predominant stromal cell is a wild-type fibroblast with functional TSC proteins [29] (Fig. 2.2). These LAM-associated fibroblasts (LAF) can be recruited by LAM-cell-derived chemokines, including stromal-cell-derived factor (SDF/CXCL12) and its cognate receptor, CXC chemokine receptor type 4 (CXCR4). The LAM cell/LAF association protects both cell types from apoptosis [29]. As in other 'tumor microenvironments', inflammatory cells are present in LAM nodules [33] including macrophages and mast cells [33, 34].

LAM is characterized by progressive lung cyst formation and lymphatic abnormalities. Cystic remodelling of the lung parenchyma leads to pneumothorax and

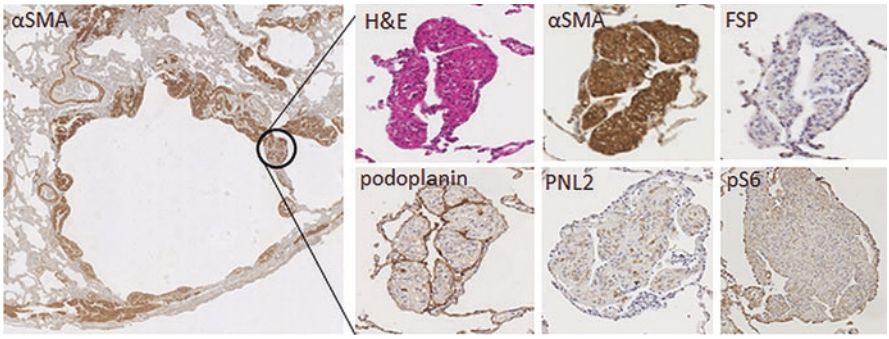

Fig. 2.2 LAM nodules contain multiple cell types. LAM nodules, identified by expression of α-smooth muscle actin (α-SMA), are composed of multiple cell types expressing fibroblast-specific protein (FSP), lymphatic endothelial cell markers (podoplanin), melanoma antigens (PNL2) on LAM cells and dysregulated mTOR signalling shown by phospho-S6 (pS6) staining

respiratory failure [5]. Lymphatic obstruction leads to chylous pleural effusions, chyloptysis and ascites [6, 35]. The mechanism of cyst formation is not fully understood, although the infiltrated LAM cells produce degrading proteases, including matrix metalloproteinases (MMP), which have been implicated in cyst formation [27]. MMPs are a family of zinc-dependent endopeptidases which were initially classified by their role in the basement membrane and ECM degradation during normal tissue turnover and growth [36]. The MMPs also have roles in the regulation of growth factors, chemokines and their ligands, inflammation and angiogenesis [37]. LAM nodules express MMP-2, MMP-9, MMP-1, MMP-13 and MMP-14 and have a reduced level of the MMP inhibitors Tissue Inhibitor of Metalloproteinases (TIMPs) 1 and 3 [38, 39]. Women with LAM have higher levels of MMP-2 in tissue and higher levels of MMP-9 in serum and urine than control women. However, two small clinical trials of the tetracycline antibiotic, doxycycline, an inhibitor of several MMPs, showed no benefit on lung function despite suppression of urinary MMP-9 [40–42], suggesting that doxycycline is not a suitable drug to target MMPs in LAM (or that other proteases are involved in the lung destruction) [42]. The serine protease plasmin is also increased in LAM lung, and its inhibitor, plasminogen activator inhibitor (PAI)-1, is reduced, as a consequence of high expression of serum response factor (SRF), leading to a proproteolytic environment [43].

Expression of another protease, cathepsin K, has been reported in LAM lesions and together with MMPs could contribute to degradation of collagen and elastic fibers [44]. Cathepsin K is a lysosomal cysteine protease predominantly expressed in osteoclasts as a bone-remodelling protease [45] and, unlike the MMPs and plasmin, is not present in normal lung parenchyma, but it is strongly expressed in LAM lung nodules [46]. Cathepsin K is also expressed in other PEComas [44], basal-like breast cancers [47] and tumor stromal fibroblasts and has been linked with tissue destruction in animal models of emphysema [48]. Cathepsin K is a potent elastase and collagenase but also selectively processes the inflammatory chemokines CXCL1, CXCL2, CXCL3, CXCL5 and CXCL8 which contain the ELR motif, enhancing their chemotactic activity and suggesting a potential role in inflammatory cell recruitment [49]. Cathepsin K is produced as a 329 amino acid proenzyme (pro-cathepsin K), which is cleaved into a 215 amino acid active form. This cleavage event requires low pH and generally occurs in the bone resorption lacunae [50]. In the cell, this usually takes place in lysosomes [51]. In tumor stroma, pro-cathepsin K activation is dependent on an acidic extracellular pH generated by membrane proton transporters, including carbonic anhydrases (CAs), vacuolar-type H^+-ATPases and sodium bicarbonate co-transporters [52]. In vitro, $TSC2^{-/-}$ cells acidify their environment in an mTOR-dependent fashion by utilizing aerobic glycolysis (also known as Warburg metabolism), which generates lactic acid, and increasing hydrogen ion exporter expression, resulting in low extracellular pH and cathepsin K activation [46]. Thus cell-cell interactions in the LAM microenvironment generate a

proteolytic environment [46]. Inhibition of cathepsin K or extracellular acidification may therefore represent a potential therapy for LAM. In bone and LAM lung, cathepsin K expression is partially mTOR dependent [53], raising the possibility that inhibition of mTOR and cathepsin K may have synergistic effects on lung destruction in LAM [46].

Whilst the mechanism underlying lung cyst formation in LAM is not completely understood, various disease-associated factors suggest that, in addition to mTOR activation, sex steroids and wild-type cells – including infiltrating immune cells – are involved in cyst formation. The gender and age prevalence of the disease implies the involvement of female hormones in LAM development and progression. Large epithelioid LAM cells around the periphery of nodules express progesterone and estrogen receptors [38]. High levels of estrogen during pregnancy [10] and in those using hormone replacement treatment [54] are associated with disease progression in LAM, and lower estrogen levels postmenopausally are associated with slower progression. In $TSC2^{-/-}$ 621–101 angiomyolipoma (AML)-derived cells, estradiol activates ERK2 to stimulate and to increase proliferation, migration and invasion [55]. Estrogen stimulation can also decrease apoptosis in vitro, by reducing BCL-2-interacting mediator of cell death (BIM) [56]. There is, however, no definite benefit of estrogen inhibition treatment in LAM [57–59]; two studies have shown no advantage in lung function decline and a significant reduction in the diffusing capacity of the lungs for carbon monoxide (DLCO) in the treated patients compared to untreated controls [10]. Moreover, the risk of osteoporosis and cardiovascular complication was increased by estrogen suppression [60]. A clinical trial of aromatase inhibition in postmenopausal women with LAM suggested that aromatase inhibition may be associated with slower loss of FEV1 [61].

The mTOR inhibitor rapamycin (sirolimus) has become the standard therapy for progressive lung disease in LAM [62]. Rapamycin treatment reduces loss of FEV1 in those treated although it may be less effective in those who have had the disease for longer and have lower pretreatment lung function [62], possibly consistent with mTOR-independent wild-type cells progressively accumulating and contributing to cyst formation.

Tumor-infiltrating immune cells, such as lymphocytes (TILs), play important roles in tumorigenesis [63]. The immune system targets and eliminates early malignant cells, but tumors may escape this immune surveillance by modulating T-cell activity. A number of checkpoints allow the immune system to promote protective immunity whilst reducing potentially harmful autoimmunity. Tumors can use these co-inhibitory pathways to prevent immune attack and elimination [63]. Targeting the immune checkpoint proteins or co-inhibitory receptors on T-cells, programmed cell death-1 (PD-1), PD-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) is effective in cancer immunotherapy [63]. In models of LAM, blocking either PD-1 or CTLA-4 delays tumor growth and increases long-term survival in animals [64], suggesting a potential role of the immune system in disease progression and possibly cyst formation in LAM.

Pulmonary Langerhans Cell Histiocytosis

Pulmonary Langerhans cell histiocytosis (PLCH) (previously eosinophilic granuloma or histiocytosis X) is a rare interstitial lung disease characterised by peribronchiolar lesions containing activated dendritic cells (DC) known as Langerhans cells (LC). PLCH can occur in isolation or, less frequently, as part of a multisystem syndrome such as Hand-Schüller-Christian disease or Letterer-Siwe disease. The clinical presentation of the disease is variable and can include cough, dyspnea and pneumothorax but is sometimes an incidental finding. The disease may progress aggressively to respiratory failure although in many cases it exhibits a favorable clinical course, sometimes with spontaneous remission [65]. The precise prevalence of the disease is unknown because around one-quarter to one-third of affected individuals are asymptomatic, but it has been estimated at 0.27 and 0.07 per 100,000 in males and females, respectively [66].

PLCH can affect both children and adults; in adults, the disease generally affects both males and females aged 20–40 years and is almost exclusively associated with cigarette smoking or second-hand exposure to cigarette smoke [66–70]. On HRCT, nodules and cysts are evident in the upper and middle regions of the lung. The disease does not generally extend beyond the lung, but some patients develop bone or skin lesions or diabetes insipidus due to pituitary involvement. PLCH can be diagnosed histologically from a surgical lung biopsy or transbronchial biopsy. Langerin, CD1a and S-100 antibodies have been used in the immunohistochemical diagnosis of PLCH; langerin/CD207 and CD1a are specifically expressed in LCs [71], which also display characteristic cytoplasmic inclusions known as Birbeck granules.

Langerhans cells are a subtype of bone marrow-derived dendritic cell normally found in the skin and the bronchial epithelium. Their function in the lung is to process and present inhaled antigen followed by migration to the lymph nodes, where they mature and interact with T-cells, promoting immunity or tolerance [72]. Smoking causes Langerhans cells to accumulate in the lungs, along with T-lymphocytes, macrophages, monocytes and eosinophils. Loose nodules of inflammatory cells 1–10 mm in diameter appear in the lung parenchyma and around the bronchioles in the upper-middle portion of the lung. As the disease progresses, localized tissue destruction occurs, generating cysts.

Although the clinical presentation of the disease is not typical of cancer, there has been controversy as to whether PLCH is a reactive inflammatory disease or a neoplasm. Willman et al. and Yu et al. [73–75] demonstrated clonal proliferation of Langerhans cells in LCH, supporting the hypothesis that the disease is neoplastic. Weintraub et al. [76] subsequently showed elevated expression of p53, which can be associated with dysregulated proliferation in cancer.

A number of groups have reported frequent somatic BRAF mutations in LCH cells [77–80]. BRAF is a cytoplasmic protein kinase downstream of receptor tyrosine kinase signalling (RTK), between RAS and MEK in the MAPK signalling pathway (Fig. 2.3). The BRAF mutation most commonly found in PLCH (V600E, found in 35%–57% of patients [81]) is an activating mutation and causes dysregulated

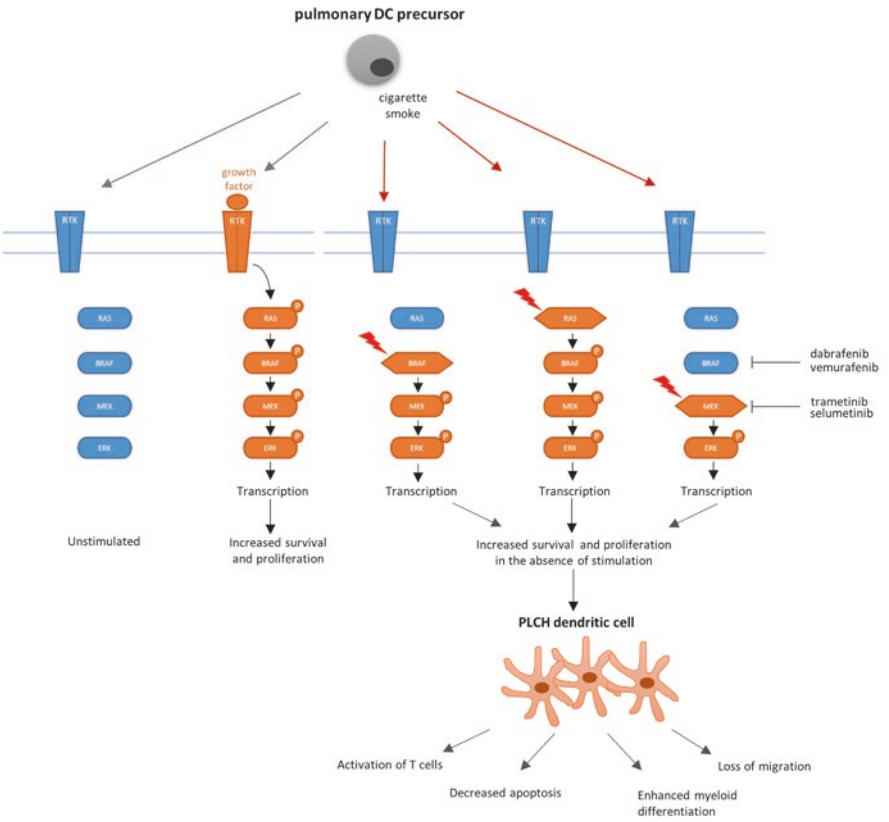

Fig. 2.3 BRAF/MEK/ERK pathway activation in Langerhans cell histiocytosis (LCH). Activating mutations in either BRAF, RAS or MEK result in ERK activation and increased dendritic cell survival and inactivation in LCH. The abnormal activation is potentially sensitive to inhibitors of BRAF and MEK

stimulation of this pathway. The RAS-RAF-MAPK pathway controls cell cycle regulation, cell proliferation, cell survival and apoptosis, and BRAF mutations have been implicated in several cancers, including melanoma, lymphoma and cancer of the lung, thyroid and colon [82–87]. Mutations in other members of the same signalling pathway have also been noted in PLCH, including *ARAF* [88], *MAP2K1* [89, 90] and *NRAS* [91]. In a recent study [91], 50% of PLCH lesions carried $BRAF^{V600E}$ mutations, and 40% harbored $NRAS^{Q61K/R}$ mutations, but these mutations were found in different clones of cells within the lesion. There is still controversy as to whether the presence of these mutations in PLCH unequivocally defines the disease as a neoplasm, given its ability to remit and resolve, but a neoplastic mechanism is currently favored.

Unlike systemic LCH, PLCH has a clear trigger, being strongly associated with cigarette smoking. Cigarette smoke causes recruitment of dendritic cells into the airways: this is an immediate response and has been observed in both mouse

models and human subjects. Cigarette smokers also display increased numbers of Langerhans cells in their airways and parenchyma [92]. Soler et al. showed a 30-fold increase in the number of Langerhans cells in the alveolar parenchyma of smokers compared with nonsmokers [93]. The mechanism by which this rapid recruitment occurs is currently unknown but may involve the induction by cigarette smoke of cytokines such as TNFα, GM-CSF, TGFβ and CCL20 in the airways.

It is not clear why LCs subsequently accumulate in the airways and parenchyma and do not migrate towards lymphoid organs. Cells harboring *BRAF* mutations could have a proliferative advantage leading to exuberant clonal expansion or could display decreased apoptosis as has been noted in melanoma and thyroid cancer. Consistent with this, Marchal et al. reported very low levels of apoptosis in the PLCH lesions and high levels of expression of the anti-apoptotic protein Bcl-xL [94]. Alternatively, trafficking to lymph nodes may be affected by altered expression of chemokine receptors such as CCR6 and 7. The consequence of this unusual behavior is the formation of characteristic PLCH lesions, containing LC, and a variable association of lymphocytes, eosinophils, fibroblasts, neutrophils, plasma cells and multinucleated giant cells. The phenotype of the LC in PLCH lesions is more typical of mature lymphostimulatory DC, normally found in the lymphoid organs, than the immature cells normally found in the airway – these mature cells are capable of initiating a significant immune response [95], perhaps targeted at the large numbers of T-cells expressing CD154 found in these lesions, a ligand expressed only transiently after T-cell activation.

As PLCH lesions are centered around bronchioles, the cysts may originate as enlarged bronchiolar lumina. However, Fukuda et al. performed a detailed ultrastructural study of early- and late-stage PLCH lung tissue and showed that around these PLCH lesions, alveolar epithelial cells show loss of attachment, their basement membranes become denuded and there is some evidence of myofibroblast recruitment in the alveolar lumen [96]. As the disease progresses, airspace enlargement and fibrosis continue; however, the contribution of S100-positive LC to the lesions appears to diminish (Fig. 2.4). There is evidence of elastin degradation and basement membrane fragmentation around the lesions leading to the suggestion that dysregulated ECM protease expression by stimulated immune cells is responsible for the breakdown of alveolar integrity. Colombat et al. also reported that almost all basement membranes had disappeared in the cyst walls and detected expression of MMP-1, MMP-2, MMP-9, MMP-12 and MMP-14 in the PLCH lesions [97]. Landi et al. used a proteomic approach to analyse bronchoalveolar lavage fluid protein composition of patients with PLCH and of controls and identified proteolytic fragments of plasma proteins (including kininogen-1 N fragments and haptoglobin) in PLCH, also suggestive of increased proteolytic activity [98].

As most patients with systemic PLCH carry a somatic activating mutation in one of the steps of the RAS-RAF-MEK-ERK signalling axis, this pathway offers an attractive target for therapy. Several reports indicate that, for patients with an identified $BRAF^{V600E}$ mutation, targeted therapy with vemurafenib, an inhibitor of mutated BRAF, results in significant clinical improvement. However, for many patients with PLCH, rather than systemic LCH, cessation of smoking results in resolution of the disease, although lung function may continue to decline as a consequence of other

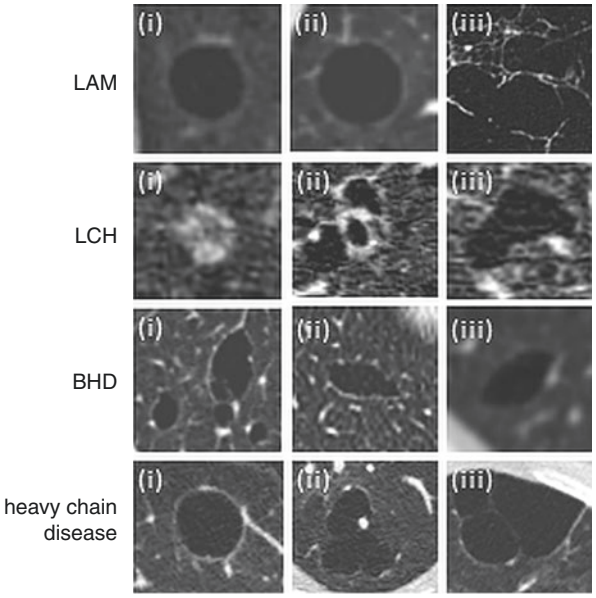

Fig. 2.4 Lung cyst morphology. Cyst characteristics among the DCLDs vary and are related to the mechanism of cyst formation. LAM: (i, ii) Cysts are round with smooth, thin walls, probably reflecting nodules surrounding cysts. Cysts are surrounded by normal lung parenchyma. (iii) In extensive disease, cysts abut each other with little intervening normal lung. PLCH: (i) Inflamed small bronchioles form nodules which cavitate. (ii) Cavitating nodules form thick-walled cysts in active disease. (iii) Later in the disease, cyst walls become less prominent, leaving irregularly shaped lucencies. BHD: (i, ii, iii) Lung cysts tend to be ovoid or lenticular, consistent with the concept that shearing mechanical forces tear apart weakened alveolar septae. Heavy chain disease: Lung cysts associated with abnormal immunoglobulin deposits have varying morphologies (i) and may be round or (ii, iii) multiple, septated and traversed by vessels

smoking-related diseases, such as COPD. Although no drug is currently approved for treatment of PLCH, cladribine, a drug used to treat the childhood form of LCH as well as multiple sclerosis and some forms of leukemia, has been reported to lead to improvement in some patients with progressive cystic PLCH [99, 100].

Birt-Hogg-Dubé Syndrome

Birt-Hogg-Dubé syndrome (BHD) is a rare autosomal dominant condition first described by Arthur R. Birt, Georgina Hogg and W. James Dubé in 1977 [101]. The disease has no gender predisposition and is characterised by fibrofolliculomas (benign hair follicle tumors) on the head and neck, pulmonary cysts and spontaneous pneumothorax. Patients with BHD can also develop kidney tumors and have a sevenfold higher lifetime risk of renal cell carcinoma than the general population [102, 103].

Pulmonary cysts in BHD are the first manifestation to appear, in early to mid adulthood, and can occur in the absence of skin and renal lesions [117]. They affect at least

80% of patients, although they do not usually affect lung function and do not appear to be correlated with smoking history. The cysts are usually small (<1 cm in diameter), bilateral, oval or irregularly shaped and in contact with the pleural surface [118–120]. They can contain residual alveolar septa, fibroblasts and lymphocytic infiltration, and it has been reported that epithelial cells, including alveolar type 2 (AT2) cells, line the cyst wall [121, 122]. Kumasaka et al. studied 229 cysts from 50 patients and noted that the cysts are bounded by normal alveolar walls, abut interlobular septa and do not contain unusual cells [118] or evidence of neoplasia (Figs. 2.4 and 2.5). In 24%–38%

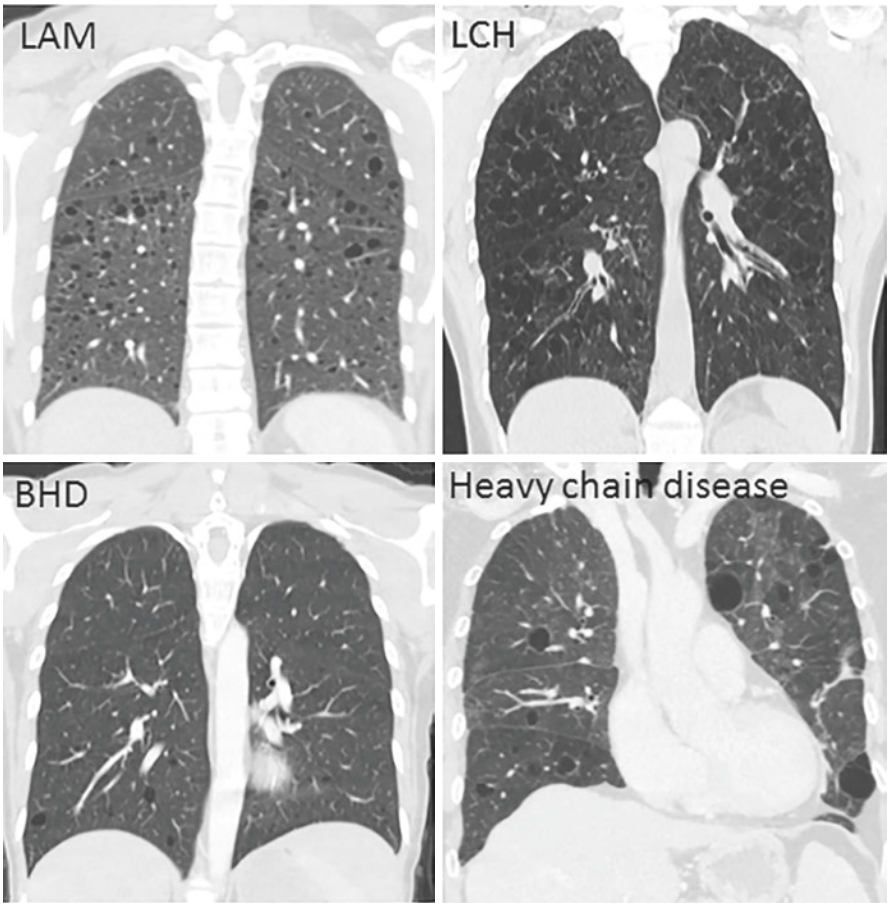

Fig. 2.5 Lung cyst distribution in specific diseases. Although speculative, it is likely that cyst distribution is related to etiology. LAM: LAM cells metastasize through blood and lymphatics resulting in a fairly homogenous distribution, perhaps tending to spare the extreme apices. PLCH: Cysts are more profuse in the upper and mid zones, perhaps reflecting the distribution of inhaled toxins including cigarette smoke. BHD: Cysts tend to be close to pleural surfaces in the mid and lower zones adjacent to the mediastinum where shear forces caused by respiratory motion are greater. Heavy chain disease: Lung cysts are randomly distributed in diffusely abnormal lung parenchyma consistent with diffuse plasma cell infiltration

of cases, they can rupture, causing pneumothorax, although the risk of pneumothorax decreases with increasing age.

In 2001, the affected locus was mapped by genetic linkage analysis to chromosome 17 and the following year to a novel gene, *folliculin (FLCN)* [104, 105], which encodes a 64 kDa cytoplasmic protein. Most of the over 100 *FLCN* mutations identified in patients with BHD are truncating, resulting in loss of function [106]. Folliculin behaves as a tumor suppressor; loss of heterozygosity of *folliculin* was identified in BHD renal lesions [107], and *flcn*+/− mice develop kidney tumors (but do not develop lung cysts) [108–110]. Fibrofolliculomas, however, do not necessarily show loss of heterozygosity of *FLCN* and may represent a haploinsufficiency phenotype [111]. The lung cysts do not appear to harbor any abnormal cells, but the high penetrance of this phenotype is also consistent with haploinsufficiency.

FLCN protein interacts with two proteins (folliculin-interacting proteins 1 and 2), which in turn interact with 5′-AMP-activated protein kinase (AMPK) [112–114]. AMPK responds to lowered intracellular ATP levels, for example, when nutrients are low, and has multiple downstream targets including the TSC2 protein, tuberin. Thus, LAM and BHD potentially share a common dysfunction – activation of the mTOR – containing protein complex MTORC1. Medvetz et al. also discovered a physical interaction between folliculin and an armadillo repeat-containing protein, plakophilin/p0071 [115]. Plakophilin is present in adherens junctions and interacts with E-cadherin, implicating loss of FLCN function in cell-cell adhesion [115, 116].

Folliculin has a widespread distribution [123]. In the lung, it is expressed in stromal cells, macrophages and alveolar epithelial cells. It has been proposed that loss of function of BHD in these cells leads to cyst formation [123], perhaps by disrupting interactions between affected epithelial and mesenchymal cells in the lung [121]. Goncharova et al. deleted *FLCN* in AT2 cells in mouse lung and demonstrated AT2 cell death, loss of epithelial integrity and airspace enlargement [124]. These effects appear to be mediated by AMPK; the knockout cells had decreased phospho-AMPK, and their phenotype was ameliorated by molecular or pharmacological AMPK activation.

As most cysts in BHD are subpleural, Graham proposed that loss of folliculin function specifically in the subpleural growth zone of the lung resulted in a failure of repair mechanisms in the lung parenchyma leading to structural fragility and cyst formation [117, 125]. Unlike PLCH and LAM, the absence of inflammation, unusual cell proliferation or unusual cell type associated with the cysts perhaps supports a model in which the alveolar septa are affected and rendered more fragile or less able to support cell adhesion. The ECM of the lung is deposited and maintained by lung fibroblasts, which express FLCN [123, 126], and is essential for attachment and survival of alveolar epithelial cells [127]. Hoshika et al. showed that lung fibroblasts from patients with BHD, carrying an identified FLCN mutation, showed reduced expression of the ECM protein fibronectin and transforming growth factor (TGF) beta, a growth factor which orchestrates tissue repair [126]. Knock down of FLCN in normal fetal lung fibroblasts showed a similar reduction in TGF beta and fibronectin expression. Both the BHD fibroblasts and the knockdown fibroblasts displayed lower chemotaxis and collagen gel contraction activity than wild-type

fibroblasts. As the BHD-derived fibroblasts still carried a functional copy of the FLCN gene, this is a phenotype associated with haplosufficiency. These data suggest that heterozygous BHD fibroblasts are deficient in key tissue repair functions, including the ability to migrate to the repair site and in the synthesis of matrix proteins.

There is no evidence that the cystic changes in BHD are a consequence of dysregulated proteolytic activity. Johannesma et al. [128] showed that cysts were stable in a 47-year-old male BHD patient for 44 months, with no increase in size or number. Further, most patients maintain normal lung function, and older patients are less likely to suffer pneumothorax, observations which are unlikely with a progressively destructive etiology. Johannesma et al. propose that loss of folliculin in the epithelial cells which have been reported to line the cysts increases cell-cell adhesion, consistent with the observations of Medvetz et al. [115, 128]. This leaves the cells less able to stretch, and under stress, the integrity of the epithelial layer is lost at its weakest point causing rupture. In this paradigm, small cysts coalesce into larger ones by rupture of the intervening septum, whilst subpleural cysts rupture into the pleural space causing pneumothorax. The idea that stretch-induced stress, combined with abnormal cell adhesion, is the causative agent in BHD lung cysts has been termed the 'Stretch hypothesis' [115, 128–130] and is consistent with the uneven spatial distribution of the cysts in BHD, where mechanical forces in the lungs may be greater in promoting cyst formation in the subpleural region.

Protein Deposition–Associated Lung Cysts

A number of diseases associated with paraprotein formation have been associated with cystic change in the lung, including light chain deposition disease (LCDD), myeloma, lymphoma, Waldenstrom macroglobulinemia and heavy chain deposition (see Figs. 2.4 and 2.5) [131]. LCDD is a rare disease, first described in 1976 by Randall et al. in two patients with renal disease [132]. LCDD is associated with overproduction of immunoglobulin light chains by plasma cells. The disease predominantly occurs in middle age and is twice as common in men [133], and around 75% of patients with LCDD have multiple myeloma or less commonly another lymphoproliferative disease, such as Waldenstrom macroglobulinemia or B-cell lymphoma. Patients with LCDD develop nonfibrillar, amorphous, eosinophilic proteinaceous deposits in multiple organs. Unlike amyloidosis, these protein deposits do not stain with Congo red and are composed of monotypic immunoglobulin light chains. The kidneys are the most commonly affected organ, but lesions can also occur in the liver, heart, small intestine, spleen, skin, nervous system and bone marrow; the lung is very rarely affected [134–136].

The initial reports of pulmonary LCDD (PLCDD) [137–142] described nodular light chain deposits in the lung. Later reports included other pulmonary features: cysts, airway involvement and bronchiectasis. The nodular form of PLCDD can be asymptomatic, but PLCDD can also take a more diffuse form, with a poor prognosis.

LCDD may rarely occur in an isolated pulmonary form without evidence of systemic B-cell proliferation. Colombat described three patients aged 28–33 years with a distinct severe cystic pulmonary phenotype associated with diffuse kappa light chain deposits but no renal manifestations of LCDD [143]. These patients were younger than typical LCDD patients, and their disease had progressed to respiratory failure. The cysts did not recur after the patients received a bilateral lung transplant, and blood and bone marrow examinations of these patients did not detect clonal plasma cell proliferation. The authors named this manifestation of LCDD *cystic lung related to LCDD* or CL-LCDD, and to date, there have been fewer than ten reports of this form of the disease. Colombat et al. demonstrated degradation of elastin fibers in alveoli, small airways and vessels in CL-LCDD and, to a lesser extent, loss of fibrillar and basement membrane collagens [97]. The authors proposed that macrophages accumulate in the vicinity of the light chain deposits and secrete an array of elastolytic and collagenolytic MMPs. Using in situ zymography, a technique which reveals localised proteolytic activity, strong gelatinolytic activity, consistent with high expression of MMP-2 and MMP-9, was detected in the vicinity of the light chain deposits.

Key Learning Points

- Cyst formation occurs when the balance of injurious environmental- and disease-related stimuli exceeds the repair capacity of the lung parenchyma.
- Dysregulation of discrete and specific signalling pathways, usually as a consequence of single gene mutations, induces injurious stimuli in rare cystic lung diseases.
- Disease-specific mechanisms causing lung damage include activation of the protease cathepsin K in lymphangioleiomyomatosis (LAM) that likely results in loss of extracellular matrix architecture, and defects in extracellular matrix attachment and repair capacity downstream of the folliculin gene in Birt-Hogg-Dubé syndrome (BHD).
- Rare diseases may share pathologic mechanisms with common diseases such as chronic obstructive pulmonary disease (COPD). mTOR-driven senescence in COPD limits alveolar repair, and mTOR-driven senescence may also contribute to cyst formation in LAM.

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