DR WEI-CHIN CHANG (Orcid ID : 0000-0002-1005-7815)
DR YU ZHI ZHANG (Orcid ID : 0000-0002-6134-980X)
PROFESSOR ANDREW GORDON NICHOLSON (Orcid ID : 0000-0003-3257-102X)

Article type : Original Article

Corresponding author mail id: rach881101@gmail.com

Mucinous adenocarcinoma arising in congenital pulmonary airway malformation: Clinicopathological analysis of 37 cases

Wei-Chin Chang[a,b,c,d], Yu Zhi Zhang[a,e], Janina L. Wolff[f], Sergei M. Hermelijn[g], J. Marco Schnater[g], Jan H. von der Thüsen[f], Alexandra Rice[a,e], Sylvie Lantuejoul[h], Bénédicte Mastroianni[i], Carol Farver[j], Fiona Black[k], Sanjay Popat[e,l,m], Andrew G. Nicholson[a,e]

[a]Department of Histopathology, Royal Brompton and Harefield NHS Foundation Trust, London, United Kingdom
[b]Department of Pathology, MacKay Memorial Hospital, Taipei, Taiwan
[c]Department of Medicine, MacKay Medical College, New Taipei City, Taiwan
[d]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan
[e]National Heart and Lung Institute, Imperial College, London, United Kingdom
[f]Department of Pathology, Erasmus Medical Centre, Rotterdam, The Netherlands
[g]Department of Paediatric Surgery, Erasmus Medical Centre, Sophia Children's Hospital, Rotterdam, The Netherlands
[h]Department of Biopathology, Cancer Center of Léon Bérard, Lyon, and Grenoble Alpes University, Grenoble, France
[i]Department of Medicine, Cancer Center of Léon Bérard, Lyon, France
[j]Department of Pathology, University of Michigan, Ann Arbor, Michigan, United States
[k]Department of Cellular Pathology, The Newcastle upon Tynes Hospitals NHS Foundation Trust, Newcastle upon Tynes, United Kingdom

[l]Department of Medicine, Royal Marsden Hospital NHS Foundation Trust, London, United Kingdom
[m]Institute of Cancer Research, Old Brompton Road, London, United Kingdom

Running title: Mucinous adenocarcinoma arising in CPAM

Conflict of interest: Andrew G. Nicholson has received consultancy fees from Merck, Boehringer Ingelheim, Pfizer, Novartis, AstraZeneca, BMS, Roche, and Abbvie; lecture fees from AstraZeneca; and research grant from Pfizer. Sanjay Popat has received consultancy fees from BMS, Roche, Takeda, AstraZeneca, Pfizer, MSD, EMD Serono, Guardant Health, Abbvie, Boehringer Ingelheim, OncLive, Medscape, Incyte, Paradox Pharmaceuticals, Blueprint, and Lilly. Sylvie Lantuejoul has received consultancy fees from MSD, AstraZeneca, BMS, and Lilly. Wei-Chin Chang has received consultancy fees from Boehringer Ingelheim. The remaining authors declare no conflict of interest.

Word count: 2544

Aims

Mucinous adenocarcinoma arising in congenital pulmonary airway malformation (CPAM) is a rare complication, with little known about its natural course. We describe a series of mucinous adenocarcinomas arising from CPAMs and present its clinicopathological features, genetics, and clinical outcome.

Methods and results

Thirty-seven cases were collected within a 34 year period, and the subtype of adenocarcinoma and CPAM, tumour location, stage, growth patterns, molecular data, and follow-up were recorded. The cohort comprised of CPAM type 1 (n=33) and type 2 (n=4). Morphologically, 34 cases were mucinous adenocarcinomas (21 in situ, 13 invasive), while 3 were mixed mucinous and non-mucinous adenocarcinoma. Seventeen cases showed purely extracystic (intraalveolar) adenocarcinoma, 15 were mixed extracystic, and 5 showed purely intracystic proliferation. Genetically, 9 of 10 cases tested positive for *KRAS* mutations, 4 with exon 2 G12V and 5 with G12D mutation. Residual disease on completion lobectomy was observed in two cases, and three cases recurred 7, 15, and 32 years after original diagnosis. Two patients died of metastatic invasive mucinous adenocarcinoma.

Conclusions

Most cases of adenocarcinomas arise in type 1 CPAMs, are purely mucinous and have early stage disease. Intracystic proliferation is associated with lepidic growth, absence of invasion, and indolent behaviour, whereas extracystic proliferation may be associated with more aggressive behaviour and advanced stage. Most cases are cured by lobectomy and recurrence/residual disease seems associated with limited surgery. Long-term follow-up is needed as recurrence can occur decades later.

Keywords: Mucinous, Adenocarcinoma, Congenital pulmonary airway malformation, Prognosis, *KRAS*

Introduction

Congenital pulmonary airway malformation (CPAM) is a rare cystic lesion composed of architecturally disordered respiratory structures, with the latest reported incidence of 1/2500.[1] Stocker originally described three histological subtypes,[2] later expanding to five subtypes,[3] although type 0 is more commonly accepted as acinar dysplasia and type 4 CPAMs are now viewed as regressed pleuropulmonary blastomas by some.[4] Type 2 CPAM is the most common,[5] and is composed of bronchiole-like cysts smaller than 2 cm in cyst size surrounded by underdeveloped/simplified alveolar parenchyma. They are often associated with pulmonary sequestrations.[5,6] Type 1 is rarer and composed of one or more cysts larger than 2 cm in diameter, accompanied by smaller cysts and alveolar parenchyma similar to that in type 2 CPAMs.

A proliferation of cytologically bland mucinous cells within the cyst lining is observed in about one-third of cases,[3] mostly in type 1 CPAMs but rarely seen in type 2 and 3 CPAMs.[5,7] These lesions have historically been classified as goblet or mucous cell hyperplasia,[8] mucinous epithelial proliferation,[9] mucinous metaplasia,[10] and atypical goblet cell hyperplasia (AGCH).[7,11,12] Occasionally, the mucinous cells also grow along the alveolar septa into the adjacent sometimes underdeveloped alveoli, microscopically indistinguishable from mucinous adenocarcinoma in situ (AIS) or invasive mucinous adenocarcinoma (IMA), dependent on size, invasion, and multifocality. With *KRAS* mutations reported in mucinous cells both within the cyst or in the adjacent alveoli,[7,9,10,13-16] and rare reports of associated metastases and mixed mucinous and non-mucinous adenocarcinoma,[17,18] these lesions are now accepted by some pathologists as neoplastic (either as AIS or IMA).

Although the nature of these lesions is better established, there is little data on outcome and follow-up of these rare lesions, even in literature reviews.[19] In this study, we describe a series of 37 cases of mucinous adenocarcinomas arising from CPAMs, looking at histopathological features (including growth patterns, nuclear features, and location of mucinous proliferation), genetic abnormalities, surgical procedures, and clinical follow-up data, with the aim of identifying key features and proposing recommendations that can be used for future management.

Materials and methods

Case selection

A review of the archives of the Royal Brompton Hospital (London, UK) and Erasmus University Medical Center (Rotterdam, The Netherlands) for adenocarcinomas resected with associated cystic changes reveal 42 cases [Royal Brompton Hospital (n=34) and Erasmus University Medical Center (n=8)] within a 34 year period, either as consultation (n=18) or routine diagnostic cases (n=24). Five cases were excluded as a result of insufficient evidence supporting the presence of cystic lesion predating tumour development and were viewed as adenocarcinomas showing cystic change as part of presentation.[20,21] The slides for the remaining 37 cases were reviewed by three thoracic pathologists (W.-C.C., A.G.N., J.H.T.), and the type of adenocarcinoma and CPAM, tumour location, location of mucinous proliferation (intracystic - defined as limited to bronchial-like or bronchiole-like cystic areas lined by mainly respiratory-type cells, or extracystic - defined as extending into alveolar-like parenchyma lined by pneumocytes), growth patterns, nuclear atypia, necrosis, lymphovascular invasion, tumour spread through airspace (STAS), pleural invasion, and synchronous atypical adenomatous hyperplasia (AAH) were recorded. In cases with more than one specimen, all had tissue from both samples available for review. Clinical data, tumour staging,[22] and follow-up were also recorded. Five of these cases (cases 12, 15, 22, 26 and 28) have been previously reported in case reports or case series[8,13,23,24].

Mutational analyses

Detection of *KRAS*, *EGFR*, and other mutations were performed on 11 cases as part of a routine clinical workup in five institutions (Royal Brompton Hospital, Erasmus University Medical Centre, Oxford University Hospitals, Cancer Center of Léon Bérard, and Centre Hospitalier Universitaire de Grenoble).

Statistical analyses

Comparisons between continuous variables were performed by one-way ANOVA test, and Fisher exact test and Chi-square test was used to assess association between categorical variables. Statistical significance is considered if p-value is <0.05 (two-sided). All statistical analyses were performed using the SPSS statistical software package (v.25.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism (v.8.3.0; La Jolla, California, USA).

Results

Clinical features

The patient cohort comprised 22 males and 15 females, and the mean age was 20.3 years (range, 0 days to 68 y), with 22 patients under 18 years of age. The main presenting symptoms/signs were recurrent infections (n=4), tachypnoea/dyspnoea (n=4), cough (n=3), pneumothorax (n=2), chest discomfort (n=3), and respiratory failure (n=2). Two infant patients were intubated immediately after birth with a suspected diagnosis of congenital diaphragmatic hernia, diagnosed by prenatal ultrasonography. The location of the cyst/tumour was available for 32 cases, with the predominant site being left lower lobe (n=10), followed by right lower lobe (n=8), left lung, not stated (n=5), left upper lobe (n=4), right middle lobe (n=3), right upper and middle lobe (n=1), and right lung, not stated (n=1). Tumour size ranged from 0.3 mm to 120 mm. Most patients underwent anatomical resections [lobectomy (n=28), bilobectomy (n=1), and pneumonectomy (n=2)], with four cases treated by non-anatomical cyst resection. One patient underwent segmentectomy followed by lobectomy when adenocarcinoma was identified in the segmentectomy. One patient underwent biopsy of a mass in the lung contralateral to the cyst.

At the time of diagnosis, tumour stages at first presentation were stage 0 (pTis; n=22), stage I (pT1 and pT2a; n=9), stage II (pT2b and pT3; n=3), and stage III (pT4; n=2). One patient (case 28) was diagnosed as stage IV (cM1a) IMA on surgical lung biopsy of mass lesions arising in a background of preceding cystic lung disease. The clinical features are summarised in Table 1.

Histopathological findings

With regard to the background cysts (Table 2), 33 were type 1 CPAMs and 4 were type 2 CPAMs, with smaller cysts lined by bronchial/bronchiolar epithelium separated by underdeveloped/simplified alveoli. In one case, mucinous adenocarcinoma was diagnosed on biopsy without sampling of the cyst, but there was unequivocal evidence of a cyst predating development of tumour.

With regard to the tumours, 34 cases were purely mucinous whilst 3 cases were mixed mucinous and non-mucinous adenocarcinomas. The majority were purely lepidic with mild nuclear atypia and no mitosis (Fig. 1A-B). Of those with invasive patterns (n=5; Fig 1C-D), all were lepidic predominant with minor acinar, papillary, or micropapillary patterns, showing mild to moderate nuclear atypia and increased mitotic activity. Two cases showed aerogenous spreading into the adjacent alveoli, resembling STAS. Synchronous AAH was observed in the adjacent lung parenchyma in 3 cases. Necrosis, lymphovascular invasion, and pleural invasion were not observed in any case. In the 4 cases where recurrence/residual tumor was observed, one (case 22) had both tumour and residual cyst in the completion lobectomy. The other three cases showed

recurrent/residual tumor only (cases 7, 17, 18). Surgical margins were not specifically sampled in the original CPAM specimen, so whether lesional tissue was present in the surgical margin could not be evaluated.

Tumour location was subdivided into involvement of cystic areas (intracystic) and extension into surrounding alveolar parenchyma (extracystic). Five cases were purely intracystic, 15 cases showed predominantly extracystic growth with a minor intracystic component (Fig. 1B), and 17 cases were purely extracystic (Fig. 1C-D; Table 2). Mixed mucinous and non-mucinous cell types, STAS and synchronous AAH were more likely to be seen in cases with purely or predominantly extracystic growth, although these did not reach statistical significance (Table 3).

Molecular findings

Molecular testing was performed in 11 cases. Nine of 10 cases tested for *KRAS* mutations were positive, and showed either *KRAS* exon 2 G12V (c.35G>T; cases 7, 9, 16, and 30) and G12D (c.35G>A; cases 28, 10, 12, 13, 18, and 28) mutations. In case 18, an additional *GNAS* p.R201H mutation was also detected alongside the *KRAS* G12D mutation observed in the original CPAM (Table 2). Four cases were tested for *EGFR* mutations and two for *ALK* rearrangement, all being negative.

Clinical follow-up

Clinical follow-up data was available for 21 cases. Residual disease on completion lobectomy was observed in two cases (cases 17 and 22), one at 7 year and one where lobectomy followed segmentectomy. Two cases recurred 32 and 15 years after initial diagnosis (cases 7 and 18), one of whom (case 7) died 45 months after M1a recurrence. One case (case 22) was found to have residual/recurrent disease 7 years after initial non-anatomical resection. One patient (case 28) died 68 months after presenting with M1a disease. Two patients (cases 2 and 4) died postoperatively, and one patient (case 37) died 130 months after diagnosis, cause unknown.

Discussion

This study shows that adenocarcinomas arising in CPAMs are typically indolent mucinous AIS or IMAs cured by anatomical resection of the cyst and surrounding lung, usually by lobectomy. However, some tumours showed more aggressive histological subtypes, and more importantly, independent of histology, can recur decades after initial resection of the cyst at an advanced stage, especially when resection is at a level less than lobectomy.

With regards to histopathological features, CPAMs with pure intracystic proliferation of mucinous adenocarcinoma cells are associated with lepidic growth, absence of invasive component, and all showed an indolent behavior with excellent prognosis, whereas CPAMs with mixed intracystic/extracystic or pure extracystic mucinous proliferations were occasionally associated with invasive architectural patterns, STAS, and tumour recurrence or intrapulmonary metastasis. This suggests that the presence of extracystic mucinous proliferations alone should be viewed as a more aggressive feature even in the absence of stromal, pleural, or vascular invasion, and a thorough examination of the resected specimen by extensive sampling may be warranted to rule out invasive components elsewhere.

Anatomical lung resection has been recommended as the optimal treatment method for CPAMs given the risk of malignant transformation.[25] In our series, there were four cases (cases 7, 17, 18, and 22; 10.8%) which had either residual disease on lobectomy or recurred years later after the initial surgery, all but one initially received non-anatomical cyst resection. The ability of these mucinous adenocarcinoma cells to spread directly into alveolar parenchyma adjacent to the the cystic areas and not be completely removed by non-anatomical resections is further exemplified by a case described by Summers et al.,[14] in which a well-differentiated multifocal mucinous adenocarcinoma arising in CPAM diagnosed in the left lower lobe wedge biopsy of an 8-year-old female was subsequently found to have residual tumour in the completion lobectomy and contralateral metastasis in the right lower lobe. Indeed, within a non-anatomical resection, it may be impossible with the naked eye at surgery or on pre-operative imaging to identify definitively the border between lesional and normal alveolar tissue outside of visible cysts. However, given that cases with non-anatomical resection in our study were mostly historical cases without adequate sampling of the surgical margins, we were unable to confirm whether the resection margins contained residual CPAM lesional tissue. Given most CPAMs are lobar, with rare exceptions,[26,27] anatomical resection would therefore seem more likely to achieve complete resection of the CPAM and any tumour therein. This is supported by a recent study of 44 infantile CPAMs with mucinous cell clusters in which resection by lobectomy was not associated with poor outcome.[27] Although numbers are small, these cases illustrate the importance of anatomical lung resection when a mucinous adenocarcinoma arising in a CPAM is identified, especially when disease extends beyond

the cyst wall. Of note, we found that AAH was also present in 8.3% of cases, and there have also been previous reports of non-mucinous adenocarcinomas with or without AAH arising in CPAMs.[28,29] These pre-neoplastic changes suggest that lung parenchyma in the vicinity of the cysts may have increased propensity to neoplastic change, and field cancerization effect might be their evolutionary origin.

There is an ongoing debate over the appropriate terminology to describe these mucinous cell proliferations, with many reports using the World Health Organization (WHO) terminology of "mucinous adenocarcinoma" (including our study),[9,10,14,16,30] while two recent studies used the terms "AGCH" and "mucinous cell clusters (MCCs)" to describe the mucinous cells.[7,27] Whilst some have argued that these proliferations should not be classified using a malignant or pre-malignant term, this seems primarily based on a lack of malignant behaviour if completely resected.[27] However, these proliferations are morphologically indistinguishable from those in adults and this study shows evidence of recurrence and metastasis if not completely resected, albeit with very slow growth rate. In addition, genetic analysis has demonstrated that these mucinous cells frequently harbor mutations of the *KRAS* gene, most commonly at exon 2 G12D (c.35G>A), G12V (c.35G >T), and G12C (c.34G>T),[7,9,10,13-16,30-32] which is similar to the *KRAS* mutations commonly found in mucinous adenocarcinomas arising de novo, typically in smokers.[33-37] In addition to *KRAS* mutation, Lantuejoul et al. also demonstrated loss of heterozygosity at the tumour suppressor genes *FHIT*, *Rb*, and $p16^{INK4}$, not only in the extracystic component but also in the intracystic component as well.[13] Consistent with the previous studies, mutations at G12D and G12V were also detected in 9 of 10 cases in our cohort, two of which eventually recurred 15 and 32 years later. Furthermore, STAS can also be observed in mucinous adenocarcinomas arising in CPAMs similar to those arising de novo,[38] although the frequency is low (2/37; 5.4%). We therefore believe that it is more appropriate to use the WHO criteria for adenocarcinoma and AIS for these lesions, although we recognise that this is an area that requires more data in the hope of achieving a consensus.[39]

The limitations of the current study are that the data are incomplete, being retrospective, spanning over 30 years and often being referred cases with limited follow-up information. Nevertheless, outcomes where data are available do provide information that help suggest management of future cases in terms of type of resection and duration of follow-up. We also have a relative lack of genetic studies, but data on those tested show recurring *KRAS* mutations, similar to those in adults with IMAs.

In summary, most cases of adenocarcinomas complicating CPAMs are purely lepidic IMAs or mucinous AISs, predominantly arising in type 1 CPAMs, although there are rare type 2 or 3 cases. CPAMs limited to intracystic bronchiolar-type proliferation are associated with absence of an invasive component and excellent prognosis, whereas those with extracystic proliferation into

alveolar tissues may be associated with more aggressive behaviour and advanced stage even in the absence of stromal, pleural, or lymphovascular invasion. Tumours share the same spectrum of *KRAS* mutations as adults with de novo IMA and further studies to look at the genetic stability of the background lung are warranted. Most cases are cured by lobectomy, but non-anatomical resection is associated with recurrence/residual disease. Long-term follow-up, the nature of which would likely be empirical and depend on factors including patient age, stage, and completeness of resection (R status), may be needed as recurrence can occur decades later.

Acknowledgements

W.C.C. co-designed the research study, analysed the data, performed the research and wrote the paper. A.G.N. co-designed the research study and gave critical review to the paper. Y.Z.Z. assisted in collecting the clinical follow-up data. J.L.W., S.M.H., J.M.S., J.H.T., S.L., B.M., C.F., F.B., and S.P. contributed the consultation cases, clinical follow-up, and molecular data. We thank Dr. Mark McCole of Oxford University Hospitals NHS Foundation Trust for his valuable case contribution. Parts of this study was presented at the 108th United States and Canadian Academy of Pathology annual meeting on March 20, 2019.

Figure legends

Figure 1. (A) Few cases show purely focal intracystic proliferation of mucinous adenocarcinoma cells adjacent to respiratory epithelium, usually in a lepidic fashion (case 13). (B) Mucinous adenocarcinoma cells growing in a lepidic pattern adjacent to a large cystic space lined by ciliated respiratory epithelium, consistent with type 1 CPAM (case 6). (C) Extracystic acinar growth set in a fibrotic stroma can be observed in some cases (case 27). (D) Extracystic papillary or micropapillary growth may be present alongside lepidic growth in some cases (case 11).

Reference

Table 1 Clinical features of mucinous adenocarcinomas arising in congenital pulmonary airway malformation

#	Age	Sex	Symptoms/Signs	Location	Treatment	Size (mm)	TNM Stage	Follow-up
1	0 d	M	Respiratory failure	LUL	Lobectomy	1.9	pTisNx	AFoD (63 mo)
2	1 d	M	N/A	Left	Lobectomy	0.4	pTisNx	Post-operative death (1 d)
3	2 d	M	Suspicious of congenital diaphragmatic hernia on prenatal ultrasound	LUL	Lobectomy	0.8	pTisNx	N/A
4	2 d	M	Respiratory failure	Left	Pneumonectomy	0.6	pTisNx	Post-operative death (12 d)
5	4 d	M	Tachypnoea	RLL	Lobectomy	1.2	pTisNx	AFoD (99 mo)
6	10 d	F	Suspicious of congenital diaphragmatic hernia on prenatal ultrasound	Left	Pneumonectomy	0.3	pTisNx	AFoD (61 mo)
7	12 d	F	Cyst resected as neonate	RUL/RML	Bilobectomy of CPAM, wedge biopsy of M1a recurrence	20	pT1bNx	M1a Recurrence at 392 mo, DoD (437 mo)
8	14 d	M	Cyst resected as neonate	LUL	Lobectomy	9	pT1aNx	N/A
9	21 d	M	N/A	LLL	Lobectomy	0.6	pTisNx	AFoD (13 mo)

10	1 mo	F	Tachypnoea, dyspnoea, cough	RML	Lobectomy	3	pTisNx	N/A
11	49 d	M	Tachypnoea, dyspnoea, respiratory acidosis	LLL	Lobectomy	0.9	pTisNx	AFoD (30 mo)
12	6 mo	M	No symptoms	RLL	Lobectomy	1	pTisNx	AFoD (385 mo)
13	2 y	M	N/A	N/A	Lobectomy	10	pT1aNx	AFoD (5 mo)
14	2 y	F	N/A	RLL	Lobectomy	1	pTisNx	AFoD (25 mo)
15	3 y	F	Recurrent infections	LLL	Lobectomy	2	pTisNx	N/A
16	4 y	F	N/A	N/A	Lobectomy	1	pTisNx	AFoD (12 mo)
17	11 y	F	N/A	N/A	Segmentectomy, then completion lobectomy	13	pT1bNx	Residual tumour in lobectomy, no further F/U
18	13 y	M	Severe chronic asthma	LUL	Non-anatomical cyst resection of CPAM, lobectomy for RLL recurrence	45	pT2bN0	Recurrence at 15 yrs (with further lobectomy), AFoD (8 mo post recurrence)[$]
19	15 y	F	N/A	RLL	Lobectomy	20	pT1bNx	N/A
20	15 y	M	Recurrent pneumothoraces	LLL	Lobectomy	2	pTisNx	AFoD (2 mo)

21	17 y	M	N/A	N/A	Non-anatomical cyst resection	2	pTisNx	N/A
22	18 y	M	History of cyst with infections	LLL	Non-anatomical cyst resection, then completion lobectomy	6	pT1aNx	Residual/recurrence at 7 yrs (with further resection), AFoD (272 mo)
23	22 y	F	N/A	N/A	Lobectomy	17 (multiple intralobar)	pT3Nx	N/A
24	27 y	F	Recurrent infections	Right	Lobectomy	17	pT1bNx	N/A
25	28 y	F	N/A	Left	Lobectomy	4	pTisNx	N/A
26	30 y	F	N/A	RLL	Lobectomy	20	pT1bNx	N/A
27	34 y	M	N/A	RML	Lobectomy	3	pTisNx	N/A
28	35 y	M	History of cyst (11 years pre-diagnosis) with infections	RML	Surgical lung biopsy of M1a disease only	15	cT3NxM1a	DoD (68 mo)
29	35 y	F	N/A	LLL	Lobectomy	3	pTisNx	N/A
30	41 y	F	Flulike symptoms, chest tightness	LLL	Lobectomy	3	pTisN0	N/A
31	42 y	M	Chest wall discomfort, cough	RLL	Lobectomy	98	pT4N0	AFoD (1 mo)

32	51 y	M	Pneumothorax	Left	Lobectomy	5	pTisNx	N/A
33	56 y	M	No symptoms	LLL	Lobectomy	120	pT4N0	AFoD (46 mo)
34	57 y	M	Dyspnoea	LLL	Lobectomy	31	pT2aN0	AFoD (115 mo)
35	61 y	M	Infections	LLL	Lobectomy	2	pTisNx	AFoD (10 mo)
36	64 y	F	N/A	RLL	Non-anatomical cyst resection	4	pTisNx	N/A
37	68 y	M	Cough, right chest pain	RLL	Lobectomy	45	pT2bN0	D cu (130 mo)

N/A: not available; RUL: right upper lobe; RML: right middle lobe; RLL: right lower lobe; LUL: left upper lobe; LLL: left lower lobe; AFoD: alive free of disease; DoD: died of disease; D cu: died cause unknown

[$] - after 4 cycles of adjuvant chemotherapy by cisplatin-pemetrexed

Table 2 Histopathological and molecular features of mucinous adenocarcinomas arising in congenital pulmonary airway malformation

#	CPAM type	Cell type	Location*	Lep (%)	Aci (%)	Pap (%)	Mic (%)	Atypia	Mitosis (/2mm^2)	STAS	AAH	KRAS
1	type 1	mucinous	extra	100	0	0	0	Mild	3	N	N	N/A
2	type 1	mucinous	extra	100	0	0	0	Mild	0	N	N	N/A
3	type 1	mucinous	extra	100	0	0	0	Mild	0	N	N	N/A
4	type 1	mucinous	extra	100	0	0	0	Mild	0	N	N	N/A
5	type 2	mucinous	extra	100	0	0	0	Mild	0	N	N	N/A
6	type 1	mucinous	extra	100	0	0	0	Mild	0	N	N	N/A
7	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	G12V
8	type 1	mucinous	extra	100	0	0	0	Mild	4	N	N	N/A
9	type 2	mucinous	extra	100	0	0	0	Mild	0	N	N	G12V
10	type 1	mucinous	extra	100	0	0	0	Mild	0	N	N	G12D
11	type 1	mucinous	intra	100	0	0	0	Mild	0	N	N	N/A
12	type 1	mucinous	intra/EXTRA	100	0	0	0	Moderate	1	N	N	G12D
13	type 1	mucinous	extra	100	0	0	0	Moderate	1	N	N	G12D
14	type 1	mucinous	intra	100	0	0	0	Mild	0	N	N	N/A

15	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	WT
16	type 1	mucinous	extra	100	0	0	0	Moderate	0	N	N	G12V
17	type 1	mixed	extra	90	5	5	0	Moderate	0	N	Y	N/A
18	type 2	mucinous	extra	50	0	30	20	Moderate	1	N	N	G12D°
19	type 1	mixed	intra/EXTRA	100	0	0	0	Moderate	0	N	N	N/A
20	type 1	mucinous	intra	100	0	0	0	Mild	0	N	N	N/A
21	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	N/A
22	type 1	mucinous	intra/EXTRA**	100	0	0	0	Mild	0	N	N	N/A
23	type 1	mucinous	intra/EXTRA	95	5	0	0	Mild	0	N	N	N/A
24	type 1	mixed	intra/EXTRA	100	0	0	0	Mild	0	N	Y	N/A
25	type 2	mucinous	extra	100	0	0	0	Moderate	0	N	N	N/A
26	type 1	mucinous	extra	100	0	0	0	Mild	0	N	N	N/A
27	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	N/A
28	type 1#	mucinous	extra	100	0	0	0	Mild	2	N	N	G12D
29	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	Y	N/A
30	type 1	mucinous	intra	100	0	0	0	Mild	0	N	N	G12V

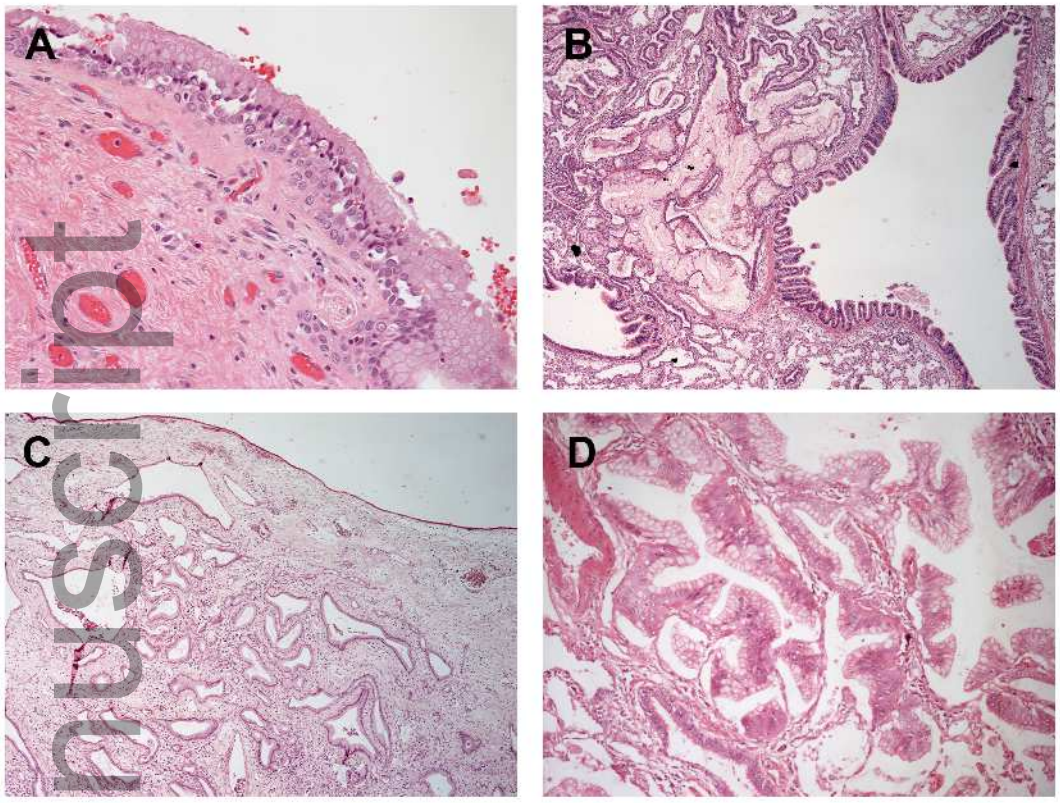
31	type 1	mucinous	intra/EXTRA	75	5	0	20	Moderate	3	Y	N	N/A
32	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	N/A
33	type 1	mucinous	intra/EXTRA	95	5	0	0	Moderate	1	N	N	N/A
34	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	N/A
35	type 1	mucinous	intra	100	0	0	0	Mild	0	N	N	N/A
36	type 1	mucinous	intra/EXTRA	100	0	0	0	Mild	0	N	N	N/A
37	type 1	mucinous	extra	100	0	0	0	Moderate	0	Y	N	N/A

*intra: tumour cells are intracystic (defined as cysts otherwise lined by respiratory-type epithelium); extra: tumour cells are extracystic (defined as extending into adjacent alveolar parenchyma lined by pneumocytes); intra/extra: tumour cells present in both areas (predominant area in capitals)
Y: yes; N: no; N/A: not available; Lep: lepidic; Aci: acinar; Pap: papillary; Mic: micropapillary; STAS: tumour spread through airspace; AAH: atypical adenomatous hyperplasia; G12D: exon 2 Gly12Asp; G12V: exon 2 Gly12Val; WT: wild type.
- based on cyst size on imaging; ** - Residual/recurrent tumour and residual CPAM in completion lobectomy; ° - associated with an exon 8 p.R201H GNAS mutation in the recurrence

Table 3 Subgroup comparison of histopathological features based on location of mucinous proliferation

Features	Intracystic (n=5)	Intracystic/Extracystic (n=15)	Extracystic (n=17)	p
CPAM type				
Type 1	5	15	13	0.071
Type 2	0	0	4	
Cell type				
Mucinous	5	13	16	0.576
Mixed	0	2	1	
Invasive component				
Absent	5	12	12	0.366
Present	0	3	5	
Nuclear atypia				
Mild	5	11	11	0.295
Moderate	0	4	6	
Severe	0	0	0	
Mitosis (/2mm^2)				
Range	0	0~3	0-4	
Mean (SD)	0 (0)	0.33 (0.82)	0.65 (1.22)	0.395
STAS				
Absent	5	14	16	0.844
Present	0	1	1	
Synchronous AAH				
Absent	5	13	16	0.576
Present	0	2	1	

AAH: atypical adenomatous hyperplasia; STAS: tumour spread through airspace



his_14239_f1.tif