Primary Mucinous (So-called Colloid) Carcinomas of the Lung

A Clinicopathologic and Immunohistochemical Study With Special Reference to CDX-2 Homeobox Gene and *MUC2* Expression

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Abstract: Herein we describe the clinicopathologic and immunohistochemical features of 13 primary mucinous (colloid) carcinomas (MCs) of the lung, an uncommon and controversial tumor. The patients, 7 males and 6 females, ranged in age from 50 to 79 years (mean, 64.5 years). All the tumors presented as a peripheral solitary nodule with gelatinous cut-surface and well circumscribed but lacking a complete fibrous wall. The size ranged from 1 to 5.5 cm. Microscopically, they consisted of neoplastic elements floating in large mucin pools and focally lining the alveolar spaces. Eleven cases were predominantly composed of tall, columnar goblet cells (goblet cell-type MC), while 2 consisted of signet-ring tumor cells (signet-ring cell-type MC). Five tumors were incidentally discovered by chest radiographs, while the others were symptomatic. All patients underwent complete surgical resection (six lobectomies and seven wedge resections). Postoperative chemotherapy was performed in 3 cases. Overall, the median follow-up was 26 months (mean 33 months; range 9–95 months). All patients with goblet cell-type MC were alive and well, while the 2 patients with signet-ring cell-type MC died of disease. Immunohistochemically, all the 11 goblet cell-type MCs were strongly stained with CDX-2 and MUC2, 8 reacted with TTF-1, 6 with cytokeratin 20 (CK20), 9 with cytokeratin 7 (CK7), and 2 with MUC-5AC. Conversely, the two signet-ring cell-type MCs were stained with TTF-1, CK7, and MUC5AC but were negative for CDX-2, MUC2, and CK20. Surfactant apoprotein-A (SP-A) was positive in four goblet cell-type and one signet-ring cell-type MC. When compared with 10 mucinous bronchioloalveolar carcinomas (m-BAC), the latter reacted with CK7, CK20, MUC5AC, TTF-1, SP-A, CDX-2, and MUC2 in 100%, 90%, 100%, 30%, 10%, 0%, and 0% of the cases, respectively. In summary, MC of the lung represents an entity with two distinct clinicopathologic and immunophenotypic variants: 1) the goblet cell-type, presenting a more indolent clinical behavior and frequently co-expressing markers of intestinal and pulmonary differentiation; and 2) the more aggressive signet-ring cell-type, which retains only markers of pulmonary origin. On morphologic and immunohistochemical grounds, MCs are easily distinguishable from m-BAC. Since goblet cell-type MC strongly stains with CDX2, MUC2, and CK20, differential diagnosis with metastatic colorectal carcinoma is very challenging and requires appropriate clinical correlation.

Key Words: lung, mucinous carcinoma, immunohistochemistry, CDX2, mucins

(*Am J Surg Pathol* 2004;28:442–452)

Primary lung tumors in which neoplastic cells float in large mucin pools are unusual, and their exact classification is still controversial. In 1978, Gowar[22] first reported the occurrence of a pulmonary multilocular cystic tumor, and additional cases were briefly described by Spencer[52] and Dail.[14] The authors originally referred to these lesions as a sort of pulmonary mucoceles, favoring the designation of cystadenoma. Since that observation, this group of tumors received various designations in the literature, including mucinous cystadenoma,[31,49] multilocular cystic carcinoma,[53] mucinous cystic tumor,[17,38] cystic mucinous adenocarcinoma,[15,26] mucinous cystic tumors of borderline malignancy,[23,41] mucinous (colloid) carcinoma (MC),[42,43] mucinous cystoadenocarcinoma,[12,16] and mucinous adenocarcinoma with signet-ring cells.[30] More recently, the WHO classification of lung and pleural tumors[54] recognized in this field three different entities: mucinous cystadenoma, MC, and mucinous cystadenocarcinoma. Briefly, WHO classification recognizes mucinous cystadenoma as "a localized cystic mass filled with mucin and surrounded by a fibrous wall lined by well-differentiated columnar mucinous epithelium." When a lesion shows "invasive growth into the surrounding lung…" and "significant atypia and prominent pseu-

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Supported by a grant of the Ministero dell'Istruzione, dell'Università e della Ricerca (Rome, Italy).

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dostratification," it is considered an MC "similar to the tumor of the same name in the gastrointestinal tract." Finally, mucinous cystadenocarcinoma is "a cystic adenocarcinoma with copious mucin production resembling tumors of the same name in the ovary, breast and pancreas".[54] However, the lack of clear-cut distinctive criteria makes their differential diagnosis difficult and somewhat arbitrary. Apart from mucinous cystadenoma (a rare mucin-rich tumor that does not seem to possess any metastatic potential), from a practical point of view, irrespective of the exact terminology and in agreement with Moran,[42,43] mucin-rich tumors of the lung should "be considered malignant in view of their potential to metastasize" even if "the malignant designation should be one of low-grade malignancy." The most important issue for the surgical pathologist actually is to distinguish whether the tumor represents a primary mucin-rich pulmonary neoplasm or a metastasis, particularly from a site where similar tumors are more likely to arise, such as gastrointestinal tract, ovary, pancreas, and breast. The clinical grounds and the radiologic findings remain essential in making this distinction, since to date no immunohistochemical studies have addressed this specific setting.

Different markers, especially thyroid transcription factor-1 (TTF-1), surfactant apoprotein-A (SP-A), and cytokeratins 7 (CK7) and 20 (CK20) coordinated pattern of expression, are extensively used in confirming the lung origin of a tumor.[10,11,32,51] Conversely, CDX2 is a highly sensitive and specific marker for differentiating colorectal carcinomas from pulmonary tumors[6,57] and is also helpful for discriminating tumors of different origin along the gastrointestinal tract when used in conjunction with different mucin types.[3,19,58] Briefly, CDX2 is a caudal-related homeobox gene encoding a nuclear transcription factor that plays a key role in the development and differentiation of fetal and adult intestinal epithelial cells.[7,18,29,47] Several works have reported that CDX2 is specifically expressed in normal epithelium of small bowel and colorectum and in the great majority of carcinomas arising in these sites.[3,5,19,37,47] Aberrant CDX2 expression is commonly seen in intestinal metaplasia occurring in stomach and esophagus.[3,5] Different studies reported CDX2 immunostaining also in gastric carcinomas with intestinal-type differentiation, in gastrointestinal carcinoids, in mucinous carcinomas of the ovary and pancreas, and in other intestinal-type adenocarcinomas occurring in different locations.[3,5,6,19,37,47,57] On the other side, mucins represent a heterogeneous group of high-glycosylated and high-molecular-weight glycoproteins encoded by several mucin genes clustered on chromosome 11p15.5, basically consisting of a common proteic backbone (apomucin) linked to oligosaccharides.[20,46] They are the major structural component of mucus and are widely expressed by most human epithelial tissues.[20,27] MUC2 and MUC5AC represent a subset of "gel-forming" mucins: the former is an intestinal-type secretory mucin mainly expressed in goblet cells of normal bowel and in intestinal adenocarcinoma, but also in intestinal metaplasia,[24,27,48,55,56] whereas the latter is mainly observed in surface mucous cells of the gastric mucosa and respiratory epithelium.[8,20,27,35,36,59] Interestingly, a significant association is reported between MUC2 and CDX2 expression.[3,58] Finally, the expression of gel-forming mucins, such as MUC2, seems to be a marker of favorable behavior in patients with pancreatic and mammary MC.[1,39,45] MUC2 expression, even if with low level, has been also previously reported in a subset of in goblet cell-type mucin-producing adenocarcinoma of the lung.[36] By contrast, MUC5AC expression in lung cancer has been reported as a marker of poor outcome[59] or in association with mucinous bronchioloalveolar carcinoma (m-BAC).[13]

Herein, we report the clinicopathologic features of 13 cases of primary MC of the lung, to address new insights about their clinical behavior and immunophenotype. In particular, we focused our attention on an immunohistochemical panel useful to identify pulmonary and intestinal differentiation (TTF-1, CK7, CK20, SP-A, CDX2, MUC2, and MUC5AC), and we evaluated its practical value in distinguishing pulmonary from extrapulmonary MC. In addition, since m-BAC is the most frequent mucin-rich tumor of the lung and shares some morphologic features with MC, but behaves differently, we compared the clinicopathologic features and the expression of the same immunohistochemical markers in a series of pulmonary m-BAC.

MATERIALS AND METHODS

The files of the Section of Pathology of the University of Modena and Reggio Emilia, and of the Operative Units of Pathology of Reggio Emilia and Mestre Hospitals were searched for cases of primary mucus-rich carcinomas of the lung. Among 5427 lung carcinomas diagnosed from 1991 to 2002 at the three above institutions, a total of 21 cases of mucinous ("colloid") carcinoma were originally collected. After a careful clinical and histologic review, 8 cases were excluded (5 were reinterpreted as a metastasis from a primary colorectal carcinoma and 3 were reinterpreted as m-BAC). Thus, a total of 13 cases (0.24% of all lung cancers) were collected and included for the current study. All the cases consisted of surgical specimens (seven wedge resections and six pulmonary lobectomies), and the tissue was routinely fixed in 10% buffered formalin and paraffin-embedded. The histologic classification was based on review of all hematoxylin and eosin-stained sections of the primary tumor (mean, 3,5 slides for each tumor; range, 2–11 slides). In addition, PAS and PAS-diastase (PAS-D) stains were performed in every case. All the slides were reviewed at a multiheaded microscope by four pathologists (G.R., B.M., A.C., L.L.) without knowledge of the clinical follow-up. The cases were included in the study if they fulfill the histologic criteria of MC, according to the WHO definition.[54] Full concordance on tumor subclassification was reached in all cases. Briefly, MC is defined as a variant of adenocarcinoma similar to that occurring in the gastrointestinal tract, consisting

of pools of mucin often distending and disrupting alveoli, in which neoplastic cells float singly or in small clusters. Based on examination of multiple hematoxylin and eosin slides, only a few foci of tall columnar cells with goblet-like features growing in a lepidic fashion were accepted and, although the alveoli resulted often was distorted by the mucoid material and thus poorly recognizable, this lepidic proliferation did not affect more than one third of the entire alveolar circumference. Epithelial pseudostratification was noted in some cases but, when occurred, it was less than 5% of the entire tumor epithelial component. Of note, all the selected cases consisted of pure MC, since none of them presented any mixture of other histologic subtypes of adenocarcinoma. The extent of necrosis (0, no necrosis; 1+, small foci of necrosis; 2+, geographic necrosis; 3+, massive necrosis occupying more than 50% of the neoplasm) and the presence of hemorrhage and lymphatic or vascular invasion were recorded.

For immunohistochemical analysis, the following antibodies were used: CK7 (clone OV-TL 12/30, Dako, Glostrup, Denmark; 1:100 dilution), CK20 (clone Ks 20.8, Dako; 1:100 dilution), TTF-1 (clone 8G7G3/1; Dako; 1:100 dilution), SP-A (clone PE-10; Medite, Milan, Italy; 1:200 dilution), CDX2 (clone 7C7/D4, Biogenex, San Ramon, CA; 1:200 dilution), MUC2 (clone M53, NeoMarkers, Fremont, CA; prediluted), and MUC5AC (clone 45M1, NeoMarkers; prediluted). In each case, 4-μm-thick sections obtained from a representative block were air-dried overnight at 37°C, then deparaffinized in xylene and rehydrated through a decreasing concentration of alcohol to water. Endogenous peroxidase activity was blocked by immersion for 10 minutes with 3% hydrogen peroxide (H_2O_2) in methanol. Sections stained with CK7 and CK20 were digested in 0.01% protease solution in 0.005 M (pH 7.6) Tris-buffered saline at 37°C for 15 minutes. For TTF-1, a microwave antigen retrieving was performed for 30 minutes in 0.01 M citrate buffer (pH 7.8). For MUC2, the sections were incubated in 10 mM citrate buffer (pH 6.0) and then boiled for 20 minutes at 98°C. Antigen retrieval was not used for SP-A and MUC5AC. Incubation with the primary antibodies was accomplished with a modified avidin-biotin-peroxidase technique using a commercial automated immunostainer (Ventana, Strasbourg, France); 3′-3-diaminobenzidine was used as the chromogene and Harris hematoxylin as the counterstain. Alveolar type II cells served as positive internal controls for TTF-1, CK7, and anti-SP-A. Appropriate sections of a primary colonic adenocarcinoma were used as positive control for CK20, MUC2, and CDX2, while normal gastric tissue was used as positive control for MUC5AC. Negative controls were included in each test by substituting the primary antibodies with nonimmune mouse IgG, at the same concentration as that of the corresponding primary antibody.

A tumor was considered positive if more than 10% of the neoplastic cells reacted, with a moderate or strong intensity, in the relevant subcellular localization (nuclear for TTF-1 and CDX2, cytoplasmic for the other antibodies).

Since it is a common view that MC (of the goblet cell-type) could represent the end of a neoplastic continuum spectrum with m-BAC and may be somewhat arbitrarily distinguishable from m-BAC, we retrieved 18 consecutive cases of surgically resected m-BAC from the files of a single institution (Section of Pathology, University of Modena and Reggio Emilia) to compare their clinicopathologic features with those of MC. All the cases of m-BAC were reviewed by three pathologists (G.R., A.C., B.M.) and reclassified according to the WHO classification.[54] Randomly, 10 of these m-BAC were also immunohistochemically tested using the above panel of antibodies.

Clinical and radiologic data of patients with MC and m-BAC were collected from pathologic reports, clinical charts, referring physicians, or directly from the patient's families. The following data were recorded: age, sex, smoking habit, presenting clinical symptoms, radiologic appearance, tumor size, location, stage, and follow-up (calculated from the date of surgery). Staging was evaluated according to the AJCC criteria.[4] The comparison between number of tumor recurrences and death related to disease in stage for stage MC and m-BAC was performed using contingency table methods. Results were tested for significance using the Pearson's χ^2 test. Differences were considered significant at P values < 0.05.

RESULTS

Clinical Findings

Clinical data of MC are summarized in Table 1. The patients consisted of 7 males and 6 females. The mean age at diagnosis was 64.5 years (range, 50–79 years). Nine patients were smokers. Radiologically, all cases of MC appeared as peripheral and solitary nodular masses having low attenuation at contrast-enhanced CT scan. All tumors were surgically resected. Six patients underwent a simple lobectomy plus regional lymph nodes sampling, while a wedge resection was performed in the remaining 7 cases. No patient had preoperative chemotherapy or radiotherapy, while 3 patients received adjuvant postoperative chemotherapy.

The tumor affected the right lung in 9 cases, while the left lung was involved in the other 4 patients. A slight predilection for the upper lobes (8 cases) was noted. In all cases, the lesion was peripherally located. Presenting symptoms consisted of cough (5 cases), hemoptysis (2 cases), and chest pain (1 case). Five patients were asymptomatic.

Eleven tumors were in stage I (5 IA and 6 IB) and 2 in stage II (1 IIA, 1 IIB). Follow-up was available for every case. Globally, the median follow-up was 26 months (mean, 33 months). Eleven patients (84.6%) were alive without disease, with a follow-up ranging from 9 to 95 months, whereas 2 patients died of tumor after 19 and 28 months. Interestingly, the

TABLE 1. Clinical Features in the MC of the Lung

Case No.	Age (yr)	Sex	Smoke	Symptoms at Diagnosis	Site	Stage	Treatment	Follow-up (mo)
1	73	F	No	Cough	RML	IB (T2, N0, M0)	L	AW (9)
2	79	F	No	Asymptomatic	RLL	IIB (T3, N0, M0)	WR + AdCh	AW (13)
3	50	F	No	Cough	LUL	IA (T1, N0, M0)	WR	AW (26)
4	53	M	Yes	Hemoptysis	RLL	IIA (T2, N1, M0)	WR + AdCh	DOD (19)
5	64	M	Yes	Cough	LLL	IB (T2, N0, M0)	L	AW (64)
6	63	M	Yes	Hemoptysis	RUL	IB (T2, N0, M0)	WR	AW (10)
7	63	M	Yes	Cough	LUL	IA (T1, N0, M0)	WR + AdCh	DOD (28)
8	73	M	Yes	Asymptomatic	RUL	IB (T2, N0, M0)	WR	AW (13)
9	59	M	Yes	Cough	RUL	IB (T2, N0, M0)	L	AW (78)
10	58	M	Yes	Asymptomatic	RUL	IB (T2, N0, M0)	L	AW (95)
11	65	F	No	Asymptomatic	LLL	IA (T1, N0, M0)	L	AW (37)
12	75	F	No	Chest pain	RUL	IA (T1, N0, M0)	WR	AW (13)
13	64	F	Yes	Asymptomatic	RUL	IA (T1, N0, M0)	L	AW (27)

MC, mucinous (colloid) carcinoma; RML, right middle lobe; RLL, right lower lobe; RUL, right upper lobe; LLL, left lower lobe; LUL, left upper lobe; L, lobectomy; WR, wedge resection; AdCh, adjuvant chemotherapy; AW, alive and well; DOD, died of disease.

patients who died had a signet-ring cell histotype and the tumor first recurred locally and subsequently metastasized to the brain and bones.

In regard to m-BAC, the relevant clinical data are tabulated in Table 2. Briefly, there were 10 females and 8 males, with a mean age of 65.6 years (range, 49–80 years). Ten patients had a remarkable smoking history. The most common radiologic pattern consisted of an ill-defined, solid peripheral nodule (8 cases), while lobar consolidation with air-bronchogram and ground glass opacity characterized the reminder 6 and 4 cases, respectively. All the patients underwent surgical resection (2 pneumonectomies, 3 bilobectomies, 12 lobectomies, and 1 wedge excision). Six patients underwent a subsequent lung resection and 9 had adjuvant chemotherapy. Tumor size ranged from 2 to 12 cm (mean, 6.5 cm) and a slight predilection for lower lobes was noted (10 cases). Only one patient was asymptomatic, while the other presented with cough (8 cases), dyspnea (4), fever (2), chest pain (1), and bronchorrhea (1). Follow-up was obtained in each case for a median of 25.5 months (mean, 32.9 months). Nine patients are alive and well, whereas 2 are alive with disease and 6 patients died of disease. Finally, one patient died of postoperative complications.

Pathologic Findings

Grossly, all the MCs were relatively well circumscribed but unencapsulated. They consisted of a peripheral nodule, gray-whitish in color, and soft gelatinous in consistency, sometimes with a central pseudocystic area. Neither necrotic foci nor hemorrhage was noted at macroscopic examination. The size ranged from 1.5 to 5.5 cm, with a mean of 2.8 cm. Histologically, 11 tumors were remarkably similar. They were relatively paucicellular and mainly consisted of large pools of PAS-D-positive, dense, and inspissated mucus filling and disrupting the alveolar spaces (Fig. 1A, B), determining several folds on the mounted slides. Generally, several hematoxylin and eosin slides were reviewed to find the very few neoplastic mucinous cells constituting the tumor. The neoplastic epithelium was represented by a single layer of tall, columnar, mucin-secreting elements admixed with goblet cells, focally lining the alveolar spaces and floating in the mucus (Fig. 1C). Of note, these monolayer sheets of mucinous cells did not completely line the alveoli but generally involved not more than one third of the entire alveolar circumference. Tumor cells showed hyperchromatic, basally oriented nuclei with occasionally prominent eosinophilic nucleoli. In a few areas, pseudostratification of nuclei and frank cytologic atypia were noted (Fig.1D). Mitotic figures were rare (mean, 1.5 mitoses per 10 high power fields [HPF]; range 1–3 mitoses per 10 HPF). We referred to these 11 cases as classic, goblet cell-type MC. The other 2 tumors showed an identical background, consisting of large pools of PAS-D positive mucoid material, but neoplastic cells floated singly or in small clusters into the mucin and only occasionally lined the alveoli (Fig. 1E). They showed a diffuse signet-ring morphology, with more pleomorphic and hyperchromatic nuclei, prominent nucleoli, and some mitotic figures (mean, 6 mitotic figures per 10 HPF) (Fig. 1F). We referred to these 2 cases as signet-ring cell-type MC. A fibrous capsule, necrotic foci, hemorrhagic infarction, and lymphangitic spread were not observed in any case.

TABLE 2. Clinical Features in the m-BAC of the Lung

Case No.	Age (yr)	Sex	Smoke	Symptoms at Diagnosis	Site	Stage*	Treatment	Follow-up (mo)
1	57	M	Yes	Fever	LL	IIIA (T3, N1, M0)†	P + AdCh	DOD (14)
2	49	M	Yes	Cough	LLL + LUL	IB (T2, N0, M0)†	L + WR + AdCh	DOD (52)
3	80	F	No	Cough	LLL + LUL	IB (T2, N0, M0)†	L + WR + AdCh	DOC (11)
4	75	M	Yes	Bronchorrhea	LL	IIB (T3, N0, M0)†	P + AdCh	DOD (34)
5	68	M	No	Cough	RUL	IA (T1, N0, M0)	L	AW (28)
6	68	F	No	Hemoptysis	LUL	IIB (T3, N0, M0)	L + AdCh	DOD (10)
7	77	F	No	Cough	RLL	IB (T2, N0, M0)	L	AW (12)
8	65	M	Yes	Dyspnea	RUL	IB (T2, N0, M0)	L	AW (38)
9	69	M	Yes	Cough	RLL/RML	IB (T2, N0, M0)	BL + AdCh	AWD (70)
10	70	M	Yes	Chest pain	RLL	IA (T1, N0, M0)	L	AW (54)
11	64	F	No	Dyspnea	RLL + RML	IA (T1, N0, M0)	WR + L + AdCh	AW (38)
12	67	F	No	Cough	RUL	IB (T2, N0, M0)	L	AW (22)
13	62	F	Yes	Fever	LLL + LUL	IB (T2, N0, M0)†	L + WR	AW (128)
14	64	F	No	Cough	RLL/RML	IB (T2, N0, M0)†	BL + AdCh	DOD (23)
15	55	F	Yes	Cough	LLL + RUL	IIB (T3, N0, M0)	L + WR + AdCh	AWD (32)
16	65	M	Yes	Asymptomatic	RLL	IA (T1, N0, M0)	L	AW (8)
17	63	F	No	Dyspnea	RUL + RML	IB (T2, N0, M0)	L + WR	AW (10)
18	64	F	Yes	Dyspnea	RUL + RML	IV (M1)	BL + AdCh	DOD (9)

mBAC, mucinous bronchioloalveolar carcinoma; RML, right middle lobe; RLL, right lower lobe; RUL, right upper lobe; LLL, left lower lobe; LUL, left upper lobe; LL, left lung; P, pneumonectomy; BL, bilobectomy; L, lobectomy; WR, wedge resection; AdCh, adjuvant chemotherapy; AW, alive and well; AWD, alive with disease; DOC, died of other cause; DOD, died of disease.

*In the cases with multiple primary tumors affecting the same lobe and in the patients who underwent double surgical resection of the lung, tumor stage refers to the major tumor at first presenttion.

†Presence of multiple foci of tumor in the same lobe.

By contrast, at macroscopic examination, m-BAC appeared as a glistening, ill-defined mucinous consolidation without distortion of the pulmonary architecture, making it somewhat difficult to define the tumor size by naked eye. Histologically, all cases of m-BAC were quite similar. The tumors consisted of a relatively monotonous proliferation of tall, columnar mucinous cells with a clear, mucin-rich cytoplasm and relatively uniform, basally oriented nuclei with mild atypia. Sometimes, the nuclei presented nuclear hyperchromasia and evident nucleoli. All these tumors were quite hypercellular and neoplastic cells presented the typical lepidic growth along intact alveolar walls, completely lining the alveolar septa but with preservation of the alveolar architecture and without invasion of the interstitial stroma (Fig. 1G, H). This neoplastic proliferation of uniform mucinous cells typically stopped abruptly determining multiple tumor micronodules and leaving irregular spaces of uninvolved lung parenchyma between the micronodules. Sometimes, tumor cells formed papillary projections and floated singly or in small clusters into the alveolar spaces. Even though mucin lakes did not represent a prominent feature, mucoid material admixed with muciphages and inflammatory cells was noted into the alveolar spaces, but no evident disruption of the alveolar structures was disclosed. As in goblet cell-type MC, mitotic rate of m-BAC was low (1–2 mitotic figures per 10 HPF).

Immunohistochemical Findings

The immunohistochemical results of MC are summarized in Table 3. As expected, in the normal pulmonary parenchyma adjacent to the tumors, TTF-1 immunostained the nuclei of alveolar pneumocytes and of scattered bronchiolar basal cells. All respiratory epithelial cells were strongly CK7 positive. Both alveolar and terminal and respiratory bronchiolar epithelial cells were positive for SP-A. MUC5AC stained the cytoplasm of scattered mucinous cells of the main bronchi and the mucin-rich cells of the associated bronchial glands. In the normal lung tissue, no staining was noted for MUC2, CK20, and CDX2.

All the 11 goblet cell-type MCs (Fig. 2A–F) strongly and diffusely stained with CDX-2 and MUC2. Immunoreactivity for TTF-1 was observed in 8 cases (72,7%), whereas CK7 and CK20 reacted in 9 (81.8%) and 6 (54.5%) cases, respectively. A moderate positivity for MUC5AC was observed in 2 cases (18%). Of note, TTF-1 staining was never diffuse but ranged from 15% to 30% of the neoplastic cells in positive cases and showed mutual exclusion with nuclear expression for CDX2

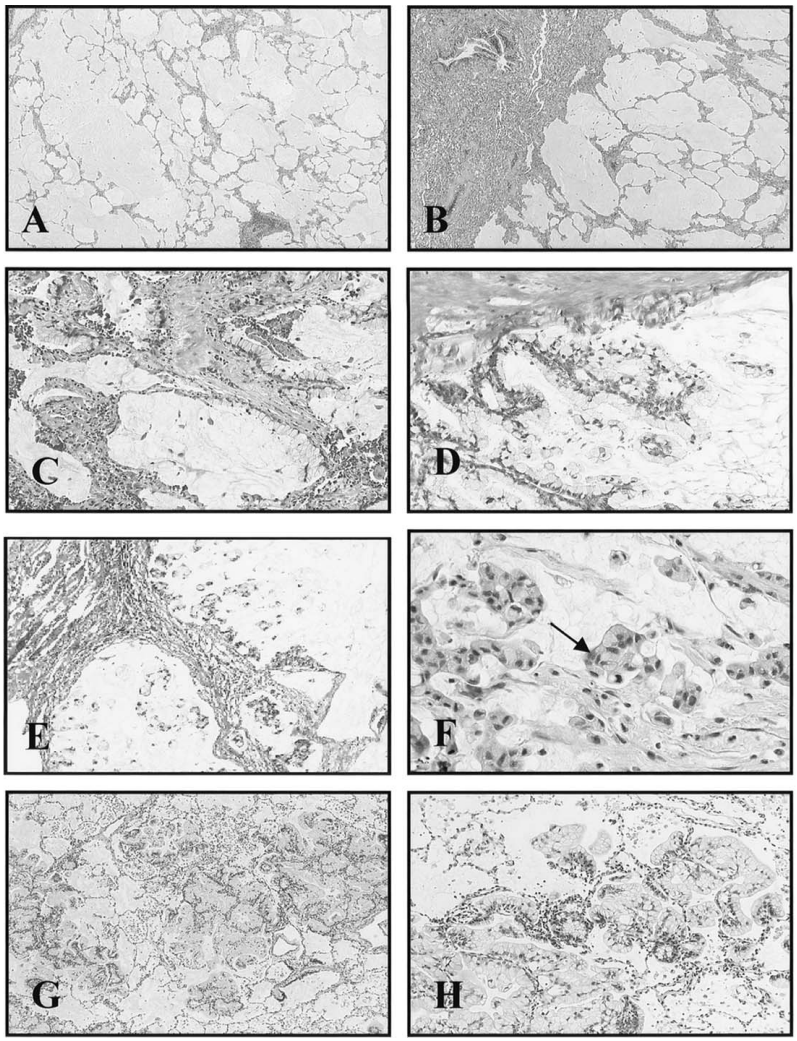

FIGURE 1. Histopathology of mucinous carcinoma of the lung. **A:** At low magnification, all mucinous carcinomas consist of abundant mucin pools distending and dissecting alveoli and (**B**) invading the surrounding lung parenchyma. **C:** Goblet cell-type mucinous carcinoma shows a neoplastic columnar mucinous epithelium lining alveoli and consisting of cells with a mucin-rich cytoplasm and basally oriented nuclei also floating into the mucus. **D:** Neoplastic elements occasionally display nuclear pseudostratification. **E** and **F:** Signet-ring cell mucinous carcinoma displays tumor cells with a clear-cut signet-ring appearance mainly floating into the mucin pools. These neoplastic elements frequently show cytologic atypia characterized by nuclear hyperchromatism and scattered mitotic figures (arrow). **G** and **H:** Mucinous bronchioloalveolar carcinoma appears as a multifocal, hypercellular neoplastic proliferation of monotonous tall columnar mucinous cells completely lining intact alveolar septa ("lepidic" growth pattern) but preserving the alveolar architecture. The tumor shows focal mucus deposition admixed with histiocytes and inflammatory cells into the alveoli.

(Fig. 2G, H). On serial sections, no tumor cells overtly disclosed both TTF-1 and CDX2 immunostaining.

Conversely, both signet-ring cell-type MCs (Fig. 3A–D) showed a strong and diffuse immunostaining for TTF-1, CK7, and MUC5AC, whereas they did not react with CK20, CDX-2, and MUC2. Finally, a moderate immunostaining for SP-A was noted in 4 goblet cell-type MC and in 1 signet-ring cell-type.

For comparison, all the 10 m-BAC were strongly positive for CK7 and MUC5AC. Nine cases stained for CK20, 3 for TTF-1, 1 for SP-A, and none for CDX-2 and MUC2.

Clinicopathologic Correlation

All MCs with CDX2/MUC2-positive expression are alive and well whatever stage at diagnosis, whereas the 2 patents with CDX2/MUC2 negative immunostaining died of disease at 19 and 28 months despite resectable tumors and adjuvant chemotherapy.

Matching stage for stage goblet cell-type MC and m-BAC, we noted that tumor recurrences and deaths related to disease were observed only in m-BAC. However, at statistical analysis, no significant differences were reached between these two groups.

DISCUSSION

MC of the lung represents a rare but distinctive variant of pulmonary adenocarcinoma. Confirming this, MC in the present series accounts for 0.24% of all lung cancers. Following the most recent WHO classification of lung and pleural tumors,[54] its diagnosis should be restricted to cases showing neoplastic cells floating in large pools of mucus and focally lining the alveolar spaces, in analogy to similar tumors arising in the gastrointestinal tract, ovary, pancreas, and breast. Although the 1999 WHO classification discriminates between MC and signet-ring cell variant of adenocarcinoma, their respective definition was not explicitly defined. Particularly, signet-ring tumor cells can be found in mucin-rich adenocarcinoma as well as in association with conventional adenocarcinoma. According to previous works,[2,30,42,44] we underline that there

TABLE 3. Immunohistochemical Results in the MC of the Lung

Case No.	Type	TTF-1	SP-A	CK7	CK20	CDX-2	MUC2	MUC5AC
1	G	+	−	−	+	+	+	−
2	G	+	+	+	+	+	+	−
3	G	+	+	+	−	+	+	−
4	S	+	−	+	−	−	−	+
5	G	−	−	+	−	+	+	+
6	G	+	−	+	+	+	+	−
7	S	+	+	+	−	−	−	+
8	G	+	−	+	+	+	+	−
9	G	−	−	+	+	+	+	+
10	G	+	+	+	+	+	+	−
11	G	+	−	+	−	+	+	−
12	G	+	+	−	−	+	+	−
13	G	−	−	+	−	+	+	−

MC, mucinous (colloid) carcinoma; G, goblet cell-type MC; S, signet-ring cell-type MC; TTF-1, thyroid transcription factor-1; SP-A, surfactant apoprotein-A; CK7, cytokeratin 7; CK20, cytokeratin 20.

seems to be two distinct types of MC: the classic goblet cell-type MC and the signet-ring cell one. The former was more frequent (11 of 13 cases). It consisted of prominent pools of mucin-disrupting alveoli and invading the adjacent lung. Columnar mucinous neoplastic elements floated into the mucus and lined the alveolar structures. Mitotic rate was very low and necrosis typically absent. Immunohistochemically, goblet cell-type MCs showed a coordinated overexpression of CDX2 and MUC2 in all cases but only partially retained the markers of pulmonary origin. In particular, TTF-1 stained 8 of 11 goblet cell-type MCs, but less intensely than in conventional adenocarcinoma.[33] The disappearance or the lack of TTF-1 expression in this type might reflect a down-regulation or switching off of the *TTF-1* homeobox gene, whereas the *CDX2* gene becomes conversely up-regulated. As previously reported in gastric carcinomas and intestinal metaplasia,[3,5] we observed an aberrant coordinated expression of MUC2 and CDX2 in goblet-type MC, confirming that CDX2 is somewhat involved in the molecular mechanism that leads to MUC2 transcription. Conversely, signet-ring type was quite more rare. As in goblet-type MC, the tumor showed abundant mucin lakes expanding alveolar spaces, but neoplastic cells, floating into the mucus or lining alveoli, had a signet-ring appearance with significant cytologic atypia. Mitoses were more frequent, but necrosis lacked. Immunohistochemically, signet-ring-type showed strong immunostaining for TTF-1 and CK7 and expresses MUC5AC, a mucin type commonly expressed in stomach and airways.[8,35,36,59]

Since the literature regarding MC is mostly composed of small series or individual case reports, it is difficult to accurately predict the behavior of this tumor. Apart from mucinous cystadenoma, an exceedingly rare tumor that does not possess any metastatic potential, it seems reasonable to consider such neoplasms (mucin-rich tumors of the lung) as a spectrum of lesions in different stages of biologic evolution and at least of low grade, then requiring a complete surgical excision together with an appropriate follow-up, as previously suggested by Moran et al.[42,43] In this regard, it is noteworthy that, in the large series by Moran et al,[42] 8 of the 19 patients with available follow-up died of disease. Also, Mann et al[38] described a cystic mucinous tumor of borderline malignancy initially treated with wedge resection that recurred 4 years later and required a subsequent lobectomy. By contrast, neither recurrences nor metastases were observed in the series of 11 lung tumors described as mucinous cystic tumors of borderline malignancy by Graeme-Cook and Mark[23] or in other similar case reports.[14–17,22,26,31,41,49,52,53] Overall, in our series of MCs, all 13 patients underwent margin-free surgical resections (six lobectomies and seven wedge resections), but 2 died of disease after adjuvant chemotherapy. In these 2 patients, the tumor recurred locally and subsequently metastasized to the brain and bones, confirming that MC possess an intrinsic malignant potential. Of note, both tumors were signet-ring cell-type MC. As more conventional adenocarcinoma with a prominent signet-ring cell component,[9,25,40] signet-ring cell-type MC seems to be related to a dismal prognosis.[2,30,44] In our view, MC should be considered at least a low-grade malignant tumor, and we prefer to use this definition to warrant an appropriate treatment, consisting of a margin-free complete resection coupled to a follow-up similar to that adopted in more conventional non-small cell lung carcinomas. At presentation, unlike other non-small cell lung cancers, different authors reported that MC were incidentally discovered.[23,42] In our series, the tumor was incidentally discovered by chest radiographs in 5 of 13 cases,

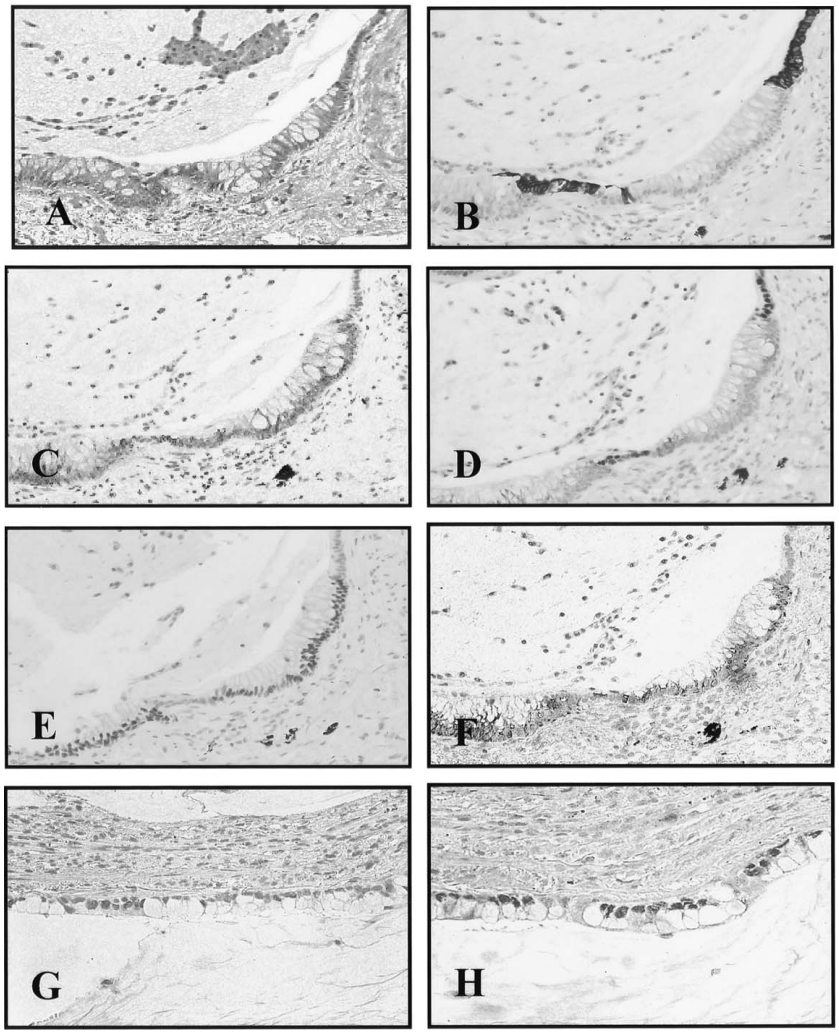

FIGURE 2. Immunohistochemical features of a goblet cell-type mucinous carcinoma. **A:** Tumor columnar mucinous epithelium lining alveolar wall alternately react with CK7 (**B**), CK20 (**C**), TTF-1 (**D**), CDX2 (**E**), and MUC2 (**F**). The peculiar up- and down-regulation of transcription factors TTF-1 (**G**) and CDX2 (**H**) is well demonstrated by the mutually exclusive immunostaining for the relevant markers.

while the other patients presented with the common symptoms of lung cancer.

Interestingly, the favorable outcome of goblet cell-type MC might be somewhat explained by the strong and diffuse expression of MUC2 in this tumor type. Indeed, this particular "gel-forming" mucin possesses specific adhesive properties related to tumor suppressor activity.[55] Supporting this hypothesis, Adsay et al[1] recently detected MUC2 expression in a series of pancreatic and mammary colloid carcinomas, two distinctive tumors usually having an indolent clinical course, but not in the more aggressive ductal counterparts.

The occurrence of a primary pulmonary neoplasm showing extensive area of mucin deposition remains a challenging differential diagnosis and essentially represents a diagnosis of exclusion. Incidentally, the most important issue lies in distinguishing a primary lung versus a metastasis from another primary site where such tumors are more likely to occur or from m-BAC, the commonest pulmonary mucin-rich neoplasm (Table 4). Since goblet-type MC is usually positive for CDX2, MUC2, and CK20, these markers appear to have no value in the differential diagnosis in regard with mucinous carcinomas from intestine. When positive, the coordinated expression of TTF-1, although less intense and diffuse than in conventional lung adenocarcinoma, and CK7 may be useful for confirming pulmonary origin.[10,11] Nevertheless, clinical and imaging investigations together with a close collaboration with the referring clinicians are clearly mandatory to formulate the definitive diagnosis. By contrast, signet-ring cell-type MC, although morphologically identical to metastatic signet-ring cell carcinoma from other sites and immunostained with MUC5AC, may be distinguished by means of immunohistochemical markers of pulmonary origin, such as TTF-1 and CK7. In agreement with previous works on primary signet-ring cell carcinoma of the lung,[9,25,40] both signet-ring-type MCs in our series strongly stained with TTF-1 and CK7, while they were completely negative for CDX2, MUC2, and CK20. Therefore,

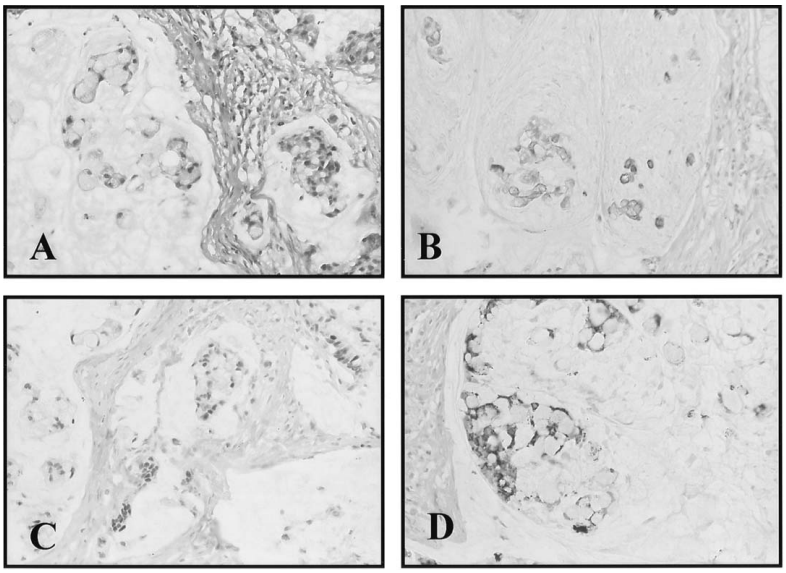

FIGURE 3. Immunohistochemical features of a signet-ring cell-type mucinous carcinoma. **A:** Signet-ring tumor cells floating into the mucus pools strongly react with CK7 (**B**), TTF-1 (**C**), and MUC5AC (**D**).

MUC5AC expression is not restricted to the gastric mucosa but also found in columnar, mucin-rich cells of the normal bronchi and bronchial glands.[35,36,59]

m-BAC is a primary lung cancer displaying similar morphology with MC (of the goblet cell-type) but a different clinical behavior, tending to disseminate through the air spaces resulting in more frequent multicentric appearance.[12] For this, it is important that MC should be distinguished from m-BAC. As observed in this work, m-BAC is quite different from MC both at radiologic and macroscopic examination, presenting as a glistening, ill-defined lobar consolidation. By contrast, MC appeared as a well-demarcated, low-density, and nodular mass exuding abundant gelatinous mucoid material. Although we failed to find any significant difference at statistical analysis, m-BAC had a poorer prognosis in comparison with goblet cell-type MC of identical stage at diagnosis, as demonstrated by the frequent occurrence of tumor recurrences and deaths related to disease we observed in the former. Histologically, m-BAC by definition shows a lepidic growth pattern characterized by a monotonous proliferation of mucin-producing, tall columnar cells completely overlining the alveolar structures, forming papillary projections into the alveolar spaces and displaying several tumor micronodules surrounded by uninvolved lung. By contrast, in MC one can need several slides to find the neoplastic mucinous epithelium that usually lines not more than one third of the entire alveolar circumference. In addition, m-BAC is more cellular than MC, and only at the periphery BAC may display mucin lakes together with a less dense neoplastic component,[12] but does not show the large pools of dense, inspissated mucus distorting and displacing the alveolar architecture, and determining several folds on the hematoxylin and eosin mounted slides, as instead happens in MC.

TABLE 4. Comparative Immunohistochemical Features of MC, m-BAC, and Metastatic Mucinous Colorectal (MCRA) and Gastric (MGA) Adenocarcinoma

	Goblet-Cell-Type MC	Signet-Ring Cell-Type MC	m-BAC	MCRA*	MGA*
TTF-1	+/−	+	−/+	−	−
CDX-2	+	−	−	+	−/+
CK7	+/−	+	+	−	+/−
CK20	+	−	+	+	−/+
MUC2	+	−	−	+	−/+
MUC5AC	−	+	+	−/+	+
SP-A	−/+	+/−	−/+	−	−

MC, mucinous carcinoma of the lung; m-BAC, mucinous bronchioloalveolar carcinoma; MCRA, mucinous colorectal adenocarcinoma; MGA, mucinous gastric adenocarcinoma; CK7, cytokeratin 7; CK20, cytokeratin 20; SP-A, surfactant apoprotein A.

*According to previous literature (references [3,5,6,10,11,19,20,24,27,29,33,34,47,56,57]).

As noted in previous papers[13,21,28,35,50] as well as in the present study, m-BAC may diffusely express MUC5AC and CK20, while they can be TTF-1 negative, similarly to goblet-type MC. However, we found that CDX2 and MUC2 may be helpful in such diagnostic setting, no m-BAC immunostaining for the above markers. Finally, it is noteworthy that normal lung structures are completely unstained with CDX2 and MUC2. Whether the finding of positive cells for the above markers in a bronchial or lung specimen should be considered as a suspicion of malignancy in broad sense (mainly including the possibility of a metastatic tumor) clearly requires further studies, mainly focusing on reactive and atypical epithelial proliferations of the lung.

In summary, we reported a detailed study on 13 cases of MC of the lung, with special interest on their immunohistochemical features. Morphologically, we observed two distinct histotypes, both sharing the presence of abundant mucin pools, but one composed of goblet cell-type neoplastic cells (goblet cell-type MC) displaying CDX2+/MUC2+ phenotype and the other consisting of signet-ring tumor cells (signet-ring cell-type MC) with a CDX2−/MUC2−/MUC5AC+ phenotype. Whether CDX2/MUC2 coordinated expression might be considered a favorable prognostic factor in pulmonary MC, as suggested in MC from other sites, clearly needs further investigations in larger series. Finally, from a diagnostic standpoint, pathologists should be aware of this aberrant expression when dealing with a mucin-rich carcinoma in the lung. On one side, coordinated expression of CDX2 and MUC2 could be helpful in selected cases for differentiating goblet cell-type MC from m-BAC, especially on small lung specimens, such as core needle biopsy or transbronchial biopsy. On the other, these markers have no value in discriminating MC from mucinous carcinomas from other sites.

ACKNOWLEDGMENT

The authors thank Mrs. Paola Manni for her skillful technical assistance in performing the immunohistochemical staining and Mr. Luca Fabbiani for production of the microphotographs.

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