Review

Surfactant proteins A and D: disease markers

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Abstract

The abundant and restricted expression of surfactant proteins SP-A and SP-D within the lung makes these collectins specific markers for lung diseases. The measurement of SP-A and SP-D in amniotic fluids and tracheal aspirates reflects lung maturity and the production level of the lung surfactant in infants with respiratory distress syndrome (RDS). The SP-A concentrations in bronchoalveolar lavage (BAL) fluids are significantly decreased in patients with acute respiratory distress syndrome (ARDS) and also in patients at risk to develop ARDS. The prominent increase of these proteins in BAL fluids and sputum is diagnostic for pulmonary alveolar proteinosis (PAP). The concentrations of SP-A and SP-D in BAL fluids from patients with idiopathic pulmonary fibrosis (IPF) and interstitial pneumonia with collagen vascular diseases (IPCD) are rather lower than those in healthy controls and the SP-A/phospholipid ratio may be a useful marker of survival prediction. SP-A and SP-D appear in the circulation in specific lung diseases. Their serum concentrations significantly increase in patients with PAP, IPF and IPCD. The successive monitoring of serum levels of SP-A and SP-D may predict the disease activity. The serum SP-A levels increase in patients with ARDS. SP-A is also a marker for lung adenocarcinomas and can be used to differentiate lung adenocarcinomas from other types and metastatic cancers from other origins, and to detect metastasis of lung adenocarcinomas. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Surfactant proteins A and D; Pulmonary fibrosis; Pulmonary alveolar proteinosis; Respiratory distress syndrome; Lung adenocarcinoma

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Abbreviations: SP-A, surfactant protein A; SP-D, surfactant protein D; RDS, respiratory distress syndrome; ARDS, acute respiratory distress syndrome; PAP, pulmonary alveolar proteinosis; IPF, idiopathic pulmonary fibrosis; IPCD, interstitial pneumonia with collagen vascular disease; BAL, bronchoalveolar lavage

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1. Introduction

Hydrophilic surfactant proteins A and D are water-soluble and belong to the collectin subgroup of the C-type lectin superfamily, along with mannose binding lectin (MBL) and collectin-43 (CL-43) [1]. The genes of lung collectins, SP-A, SP-D, and MBL have been mapped to human chromosome 10 [2,3]. The human SP-A and SP-D genes are placed at 10q22-q23 while MBL is localized at 10q21. Lung collectins are synthesized by alveolar type II cells and Clara cells within the lung. Analysis by immunoblot and reverse transcriptase-polymerase chain reaction (RT-PCR) has revealed that SP-A is expressed in the small intestine and colon but not in the stomach in the rat [4], while the presence of SP-A in human gastrointestinal tract has so far not been reported. SP-D protein and mRNA have also been detected in mucus-secreting cells of rat gastric mucosa by immunostaining and RT-PCR [5]. One report shows that human stomach but not heart or liver expresses SP-D mRNA [6], as determined by RT-PCR. Although there are several lines of evidence that pulmonary and gastrointestinal epithelium produce closely related surface-active materials, the expression of SP-A and SP-D is predominantly strong in the lung.

Alterations in pulmonary surfactant may contribute to the pathogenesis of lung diseases such as respiratory distress syndrome (RDS), acute respiratory distress syndrome (ARDS), interstitial lung diseases and lung cancer. Several studies with bronchoalveolar lavage (BAL) fluids in patients with idiopathic pulmonary fibrosis (IPF) and pulmonary alveolar proteinosis (PAP) have demonstrated abnormalities including the decreased recovery of surfactant phospholipids, decreased content of phosphatidylglycerol, and decreased ratio of phosphatidylglycerol (PG)/phosphatidylinositol (PI) [7-9]. Phospholipid analysis in amniotic fluids has also been useful to evaluate fetal lung maturity. The determination of lecithin/sphingomyelin ratio and disaturated phosphatidylcholine has been clinically practical. Primary lung adenocarcinoma is marked by a high content of saturated phosphatidylcholine, predominantly of the dipalmitoyl species [10]. Metastatic adenocarcinomas in the lung originating from another organ exhibit different phospholipid profiles from primary lung adenocarcinoma [10], suggesting phospholipid analysis is one of the approaches to differentiate lung carcinomas. The disadvantages of phospholipid analysis may include a necessity for specimen quantity and sensitivity.

The abundant and restricted expression of SP-A and SP-D within the lung makes these collectins specific markers for lung diseases. cDNAs for SP-A and SP-D and specific antibody against each protein have been shown to be useful and sensitive tools to detect SP-A and SP-D. Establishment of enzyme-linked immunosorbent assay (ELISA) for each collectin also enables the determination of the absolute amount of the proteins [11,12]. In this review article we discuss the detection of SP-A and SP-D as disease markers in various specimens. Although lung surfactant had been believed to exist solely in the lung, SP-A and SP-D have recently been detected in serum. Since the detection of lung collectins in serum is expected to be a useful and non-invasive diagnostic tool for specific
2. Enzyme-linked immunosorbent assay (ELISA)

2.1. Amniotic fluids

Respiratory distress syndrome (RDS) of newborn infants is caused by lung immaturity. Fetal lung maturity has been assessed by phospholipid analysis of surfactant in amniotic fluids [13], and microbubble stability test [14]. Determination of accurate amounts of the apoproteins using polyclonal and monoclonal antibodies against human SP-A, in combination with phospholipid analysis [11,15], enabled clinicians to predict lung maturity more precisely. We have developed a two-site immunoassay of SP-A using two monoclonal antibodies against human SP-A [16]. In normal pregnancies, the concentration of SP-A in the amniotic fluids at less than 30 weeks gestation is very low (mean 0.84 μg/ml). It then increases approximately 6.5-fold from 34 to 36 weeks of gestation and approximately 15.5-fold at 37 weeks of gestation. This ELISA assay is affected remarkably little by blood or meconium contamination. Thus, it accurately predicts lung maturity [17], although it may give a false positive result in severe preeclampsia [18]. SP-D concentrations in amniotic fluids increase progressively with increasing gestational age [12].

2.2. Bronchoalveolar lavage (BAL) fluids

2.2.1. Pulmonary alveolar proteinosis (PAP)

Pulmonary alveolar proteinosis (PAP) is characterized by accumulation of large amounts of pulmonary surfactant within the alveoli [19]. When the supernatants of BAL fluids are analyzed after low-speed centrifugation, both SP-A and SP-D levels are prominently high in BAL fluids and so are surfactant phospholipids [20,21]. SP-A concentrations in BAL fluids in patients with PAP increase more than 10-fold compared to those from healthy volunteers (39.3 ± 12.5 μg/ml vs. 3.5 ± 1.1 μg/ml). Although the SP-D concentrations (0.88 μg/ml) in BAL fluids from healthy individuals are lower than those of SP-A, the degree of increases of SP-D in patients with PAP is larger (21.9-fold, 19.3 ± 9.3 μg/ml). These studies clearly indicate that SP-A and SP-D levels in BAL fluids are diagnostic for PAP.

2.2.2. Pulmonary fibrosis

The total phospholipids recovered at lavage are reduced in patients with IPF relative to normal volunteers [9]. The concentrations of SP-A and SP-D are also significantly decreased in patients with idiopathic pulmonary fibrosis (IPF) and interstitial pneumonia with collagen vascular disease (IPCD) [21,22]. The collectin concentrations in IPF and IPCD are decreased by 30–50% compared to controls. McCormack et al. [23] have analyzed 44 IPF patients and 33 healthy volunteers, and have concluded that the mean SP-A/phospholipid ratio is significantly lower in patients with IPF than in healthy volunteers (31.8 ± 2.8 vs. 63.9 ± 0.4 μg/μmol) and in patients who died within 2 years than in those who survived (23.4 ± 2.6 vs. 37.5 ± 4.2 μg/μmol). The 5-year survival of patients with SP-A/PL above the median level for all patients with IPF is more than twice that of patients below the median (68 vs. 30%). This study shows that the determination of SP-A in BAL fluids in patients with IPF is not only a diagnostic marker but also is a biochemical marker in lavage that predicts survival when SP-A is normalized to phospholipid. Future analysis including SP-D determination will address the values and meaning of SP-A/phospholipid ratio in patients with early stage of lung fibrosis to determine if SP-A/phospholipid is a useful marker of survival at a potentially reversible phase of the illness.

2.2.3. Acute respiratory distress syndrome

Gregory et al. [24] analyzed the crude surfactant pellets recovered from BAL fluids of patients with acute respiratory distress syndrome (ARDS) and patients at risk to develop ARDS. SP-A, SP-B and phospholipids significantly decreased in patients with ARDS and also in patients at risk to develop ARDS. At-risk and ARDS patients had significantly decreased surfactant biophysical activity. This study suggests that surfactant supplementation may be a useful treatment in ARDS and that early treatment with surfactant supplementation for at-risk patients might be beneficial to the prevention of ARDS.
2.3. Tracheal aspirates

The SP-A determination in tracheal aspirates in infants with RDS is useful to monitor the secretion of endogenous surfactant. In placebo-treated infants with RDS, SP-A was low on the first day of life and increased with time as RDS resolved itself, while in infants with RDS treated with surfactant, significantly higher levels of SP-A were observed by 2 days after treatment and were maintained through at least the sixth day of life [25]. This study suggests that endogenous surfactant secretion may be stimulated by treatment with synthetic surfactant.

2.4. Sputum

One report on the SP-A determination in sputum is available [26]. Sputum samples from three PAP patients and twenty patients with other lung diseases were assayed for SP-A. SP-A concentrations in sputum from PAP patients were 400 times higher than in controls. The measurement of sputum SP-A is useful for the diagnosis of PAP and clearly less invasive in comparison with diagnostic biopsy procedure and bronchoalveolar lavage.

2.5. Pleural effusions

One cancer cell line isolated from pleural effusions of patients with lung adenocarcinoma possessed characteristics of alveolar type II cells, well-developed microvilli and cytoplasmic lamellar bodies [27]. mRNAs for SP-A, SP-B, SP-C and SP-D were also positively detected and the SP-A protein was found to be secreted in this cancer cell line [28]. Since SP-A has been immunohistochemically detected in tumors of approximately 50% of patients with lung adenocarcinomas [29], cancer cells that invade into the pleural cavity in patients with lung adenocarcinomas may produce and secrete surfactant proteins. The SP-A protein is detected by immunoblotting analysis of pleural effusions from patients with lung adenocarcinoma [30]. The SP-A concentrations in pleural effusions from patients with lung adenocarcinomas are found to be more than 10 times higher than those from lung epidermoid carcinoma, small cell lung carcinoma, adenocarcinoma of other origins and tuberculosis [30]. Since our preliminary study indicates that SP-D concentrations are also high in pleural effusions of patients with lung adenocarcinomas, the determination of SP-A and SP-D in malignant effusions could contribute to differentiating primary lung adenocarcinoma from other types of lung carcinomas and adenocarcinomas of miscellaneous origin.

2.6. Serum

2.6.1. Do surfactant proteins appear in the bloodstream?

Although surfactant components had been believed to exist solely in the lung, Chida et al. [31] suggested that surfactant proteins could be found in sera of patients with RDS using a competitive ELISA with polyclonal antibody against SP-A or SP-B. A positive result for SP-A was obtained in 4 infants with RDS at 1 week of age and 1 surfactant-treated infant. All sera obtained at 2 months of age were negative for SP-A and SP-B. The specificities of antibodies and the presence of the surfactant proteins in serum were not demonstrated in this study. Since the positive reactions were determined as relative optical density compared to control, the absolute values of the surfactant proteins in sera were not shown. SP-A has also been reported to leak into the circulation in patients with ARDS [32]. It is clear that serum proteins leak into air spaces during severe RDS [33]. Thus, it is reasonable that surfactant proteins leak into the vascular space when permeability of vessel walls increases.

The ELISA with two monoclonal antibodies (PC6 and PE10) to human SP-A has been applied to the sera of patients with interstitial lung diseases in this laboratory [34]. The sandwich ELISA is capable of determining an SP-A level ranging from 2–250 ng/ml when native SP-A isolated from patients with PAP is used as a standard [35]. Since human SP-A has been found to contain blood group A antigenic determinants [36], the criticism may be raised that monoclonal antibodies PC6 and PE10 may recognize simply group A antigen but not SP-A in the blood samples. Monoclonal antibody (MAb-8) prepared by Stahlman et al. [36] actually agglutinated red cells and immunostained a wide variety of secretory glands and epithelium other than lungs from individuals with A or AB blood group. Reactivity of MAb-8
with SP-A persisted after endoglycosidase-F treatment, but was lost after digestion with collagenase. However, both PC6 and PE10 reacted with SP-A after the digestion with endoglycosidase-F and collagenase. Epitope mapping by treatment with BrCN and lysyl endopeptidase has revealed that PC6 and PE10 recognize the peptide portion contiguous to or near the region of the small disulfide loop of human SP-A CRD [37]. The immunohistochemical recognition by PC6 and PE10 is essentially specific to lung cells [38]. When serum proteins were applied to an immunoaffinity column that monoclonal antibody PE10 was covalently linked with, the isolated protein, which reacted with anti-SP-A antibody by immunoblotting analysis, showed a band with an apparent molecular mass of 35 kDa, corresponding to the molecular size of SP-A [34]. This sandwich ELISA also showed a positive reaction with the fraction that bound to a mannose-Sepharose 6B when human serum was applied to the affinity column (Fig. 1). Immunoblotting analysis revealed that the serum protein binding to the mannose-affinity matrix was a PE10-reactive protein with an apparent molecular mass identical to that of SP-A isolated from BAL fluids (Fig. 1, inset). Since neither PE10 nor PC6 recognize human mannose-binding lectin, the protein binding to the affinity matrix is likely to be SP-A. Taken together, the data supports the idea that our ELISA can detect SP-A that exists in sera.

One study [39] has experimentally demonstrated that SP-A leaks from alveolar spaces into vessels. Human recombinant SP-A and/or artificial surfactant was intratracheally injected into immature newborn rabbits, and human SP-A in alveolar washings and sera was monitored by ELISA with PC6 and PE10, which do not crossreact with rabbit SP-A. The group that intratracheally received human SP-A and saline showed that 2.4% of the human SP-A that was instilled into the lungs was detected in sera by ELISA. Since the group receiving saline alone showed no detectable human SP-A in sera, this study demonstrates that SP-A leaks from alveolar

![Fig. 1. Mannose-Sepharose 6B column chromatography of serum from a patient with pulmonary alveolar proteinosis. Serum from an alveolar proteinosis patient was applied to a mannose-Sepharose 6B column, and the protein binding to the affinity matrix was eluted with 2 mM EDTA. Each fraction was monitored at 280 nm (c) and the absorbance at 450 nm (d) obtained by SP-A ELISA was also plotted. Inset shows immunoblotting analysis (lane b) of the fractions (Fr 24 plus 25) binding to the affinity matrix using monoclonal antibody PE10. Lane a: molecular mass standard.](image)

![Fig. 2. The serum concentrations of SP-A in patients with various lung diseases. The bar indicates the number of individuals that exhibit each range of SP-A concentration.](image)
space into the bloodstream. When surfactant replacement was performed at the same time as SP-A instillation, only 0.05% of human SP-A appeared in the circulation. This indicates that the surfactant replacement therapy may block alveolar-to-vascular leakage of surfactant protein that was presumably caused by increased permeability due to lung injury. This is consistent with the results from surfactant-treated infants with RDS [31]. Only one RDS infant with surfactant replacement therapy showed positive levels of serum SP-A, while 4 patients without surfactant replacement had higher SP-A levels. These studies imply that the serum SP-A levels may reflect the degree of lung injury in various forms of respiratory failure.

2.6.2. Serum SP-A concentrations in interstitial lung diseases

The concentrations of SP-A in 323 healthy adults at ages of 30-70 were examined by sandwich ELISA with PC6 and PE10 (Fig. 2). There was no difference in SP-A concentration among the ages. The mean value of serum SP-A was 24.6 ng/ml. There was also no difference between males and females. The previous [34,35,40] and our recent data demonstrate that the SP-A concentrations in sera from patients with PAP and IPF were prominently high: 74 ± 45.7 ng/ml (mean ± S.D., n = 11) for PAP and 67.9 ± 42.5 ng/ml (n = 112) for IPF. These values are statistically significant when compared to healthy controls (P < 0.001). The serum SP-A levels of patients with pneumoconiosis, tuberculosis, diffuse panbronchiolitis, bacterial pneumonia and chronic pulmonary emphysema increase moderately but the values are significant compared to controls. The SP-A concentration of patients with bronchial asthma and sarcoidosis are almost the same level as healthy individuals. Patients with IPCD, collagen vascular diseases that have pulmonary fibrosis as a complication, exhibit significantly higher SP-A concentrations in sera (55.3 ± 37.9 ng/ml, n = 36). In patients with collagen vascular diseases, pulmonary fibrosis is a chronic and progressive complication that may lead to death. Since it is important to detect fibrotic changes of the lung as early as possible, the determination of serum SP-A levels could be one of the screening methods to examine pulmonary complications in patients with collagen vascular diseases.

Fig. 3. The serum concentrations of SP-D in patients with various lung diseases. The abbreviations used are: HV: healthy volunteers; IPF: idiopathic pulmonary fibrosis; IPCD: interstitial pneumonia with collagen vascular disease; PAP: pulmonary alveolar proteinosis; SAR: sarcoidosis; TB: tuberculosis; BA: bronchial asthma; BE: bronchiectasis; CPE: chronic pulmonary emphysema; DPB: diffuse panbronchiolitis; PN: bacterial pneumonia.
2.6.3. Serum SP-D concentrations in interstitial lung diseases

SP-D also appears in the circulation in patients with interstitial lung diseases (Fig. 3). The mean SP-D level in serum from healthy volunteers (n = 303) is 48.7 ng/ml. Although the amount of SP-D (0.88 ± 0.13 μg/ml) recovered in bronchoalveolar lavage fluids is less than that of SP-A (3.5 ± 1.1 μg/ml) in the alveolar space [20,21], the SP-D concentration in serum is clearly higher than the serum SP-A concentrations.

Previous [21] and our recent data also demonstrate that patients with PAP, IPF and IPCD also exhibit significantly higher concentrations of SP-D in sera. The absolute values of serum SP-D in these patients are higher than those of serum SP-A; 461 vs. 74 (ng/ml) in PAP; 339 vs. 67.9 in IPF; 208.7 vs. 56.4 in IPCD. There are no significant increases of serum SP-D levels in patients with bronchial asthma, bacterial pneumonia, diffuse panbronchiolitis and chronic pulmonary emphysema.

Taken together with SP-A data, SP-A and SP-D in sera could be a useful marker for PAP and IPF, and the determination of these proteins in serum could be a useful tool to screen pulmonary complications in collagen vascular diseases.

2.6.4. Positivity of serum SP-A and SP-D

When the cut-off values (mean ± 2 S.D. of healthy control group) are set at 48.3 ng/ml for SP-A and at 109 ng/ml for SP-D, the positivity (86.2%) of SP-D in IPF patients is higher than that (71.4%) of SP-A (Fig. 4). The positivity of SP-D in PAP and IPCD is also higher than that of SP-A. Since the values of SP-D range more widely when compared to those of SP-A, SP-D may be more appropriate to use as a screening test.

2.6.5. Correlation between serum lung collectin levels and various parameters in IPF patients

There is a very weak positive correlation between serum levels of lung collectins and serum lactate dehydrogenase (LDH). Although LDH is widely used as a serum marker for IPF, serum LDH level increases in diseases other than lung diseases. Since SP-A and SP-D are lung-specific, it is clear that serum SP-A and SP-D are superior to LDH as an IPF marker. However, the serum levels of SP-A and SP-D does not appear to be correlated with the lung physiology which is evaluated by PaO₂, vital capacity and other lung functioning tests [21,35]. This may indicate that serum SP-A and SP-D do not express the severity of impairment of lung function.

2.6.6. Correlation between serum collectin levels and their disease activity

Fig. 5 shows a case with IPF in which serum levels of SP-A and SP-D were successively monitored. After receiving corticosteroid therapy, this patient exhibited improvement of symptoms and chest radiographic findings. Consistent with the improvement, the levels of serum SP-A and SP-D decreased. The levels of serum LDH appeared unchanged during this period. However, this patient died because of respiratory failure due to acute exacerbation with concomitant increasing levels of serum SP-A and SP-D.

Serum SP-D was also measured before and after steroid therapy in 4 patients with pulmonary fibrosis [21]. Three IPF patients and 1 IPCD patient who had exhibited progressive dyspnea with high levels of SP-D (489–1906 ng/ml) before therapy improved their symptoms and chest radiographic findings with marked decreases of SP-D (5–25% less than before therapy). The IPF patients who died because of respiratory failure due to acute exacerbation showed concomitant increasing concentrations of serum SP-D levels (947–2032 ng/ml, 400–788% more than before exacerbation). The serum SP-A levels of 4 IPF patients also rose markedly with deterioration of the disease before their deaths [34,35]. These...
cases suggest that the serum level of surfactant proteins reflects the disease activity of pulmonary fibrosis.

Four patients with PAP who had progressive dyspnea exhibited prominently high levels of serum SP-D (239–2137 ng/ml) [21]. After therapeutic whole lung lavages, their symptoms, chest radiographic findings and PaO2 improved with a corresponding decrease of serum SP-D (13–36% less than before lavage). The serum SP-A levels of PAP patients also decreased after therapeutic lavage with a corresponding improvement of symptoms [34].

2.6.7. Mechanisms by which lung collectins appear in the circulation

The mechanisms by which SP-A and SP-D appear in the circulation remain to be resolved. Several hypothetical ones are speculated; the alteration in the ability of surfactant production by alveolar type II cells, the increased gradient of the apoprotein concentrations between the alveoli and the circulation, the increased permeability of lung vessels, the destruction of the barrier between alveolar epithelium and endothelium based on the injury of basement membrane, and their decreased clearance rates from the circulation. Phospholipid analysis of BAL fluids of patients with IPF and PAP reveals decreases in the phosphatidylglycerol/phosphatidylinositol ratio and in the phosphatidylglycerol content [7–9]. Since alterations in phospholipid profiles of surfactant reflect alterations in the functions of alveolar type II cells, this type of cells might be involved in the appearance of SP-A and SP-D in the bloodstream in these diseases. In PAP patients, the high levels of serum SP-A correlated well with SP-D. The contents of SP-A and SP-D in BAL fluids are prominently high as well as those of total proteins and phospholipids. SP-A and SP-D, which alveolar spaces have been filled with, make the gradient of apoprotein concentrations between the alveoli and the circulation much greater in PAP patients than in healthy controls. As a result, lung collectins might be leaking out from the alveoli into the vessels in PAP patients. The increased permeability of lung vessels may result in alveolar-to-vascular leakage of surfactant proteins under such conditions as RDS or ARDS. In these diseased states serum proteins are known to leak into alveolar space from the vessels [33]. It is reasonable to speculate that a bidirectional flux of proteins occurs. It is likely that the mechanism of the appearance of SP-A and SP-D in sera of patients with IPF and IPCD is distinct from that in PAP or RDS. Unlike in PAP patients, the amounts of SP-A and SP-D in BAL fluids from patients with IPF and
IPCD are rather lower than those in healthy individuals [20,22]. Alveolar reconstruction occurs in IPF and IPCD, and as a result the basement membranes in the alveolar epithelium and vessels are injured. When the barrier between alveolar epithelium and endothelium is consequently destroyed, surfactant proteins produced by alveolar type II cells may leak into the bloodstream. In addition the clearance system of SP-A and SP-D in the circulation is also unclear and should be investigated in future studies.

3. Immunohistochemistry and molecular biology technique

3.1. Hyaline membrane disease

Monoclonal antibodies (PC6 and PE10) against human SP-A have been applied for pathological examination of the lungs of infants with hyaline membrane diseases [38]. These antibodies stained faint granules in the cytoplasm of alveolar type II cells in adult lung. A fetal lung of 20 weeks gestation had no positive staining. A few scattered positive cells were observed in a newborn lung of 31 weeks gestation, and the stained cells increased progressively with increasing gestational age. These findings correspond to the profile of SP-A concentrations in amniotic fluids at increasing gestational ages [16]. These antibodies stained very few cells in the lungs of the newborns who died of RDS, but the lungs of newborns who died of other causes after recovery from RDS showed many positively stained cells. All of these cases exhibited hyaline membranes pathologically. Hyaline membranes are a reliable pathological criterion for the diagnosis of RDS. However, hyaline membranes can also be observed in pneumonia, uremic lung, ARDS and other pathological states that cause alveolar destruction and exudation. Thus, SP-A staining with these antibodies could be a good pathological indicator reflecting the presence of surfactant and differentiating hyaline membrane diseases.

3.2. Lung adenocarcinomas

Several immunohistochemical studies have been performed to examine the expression of SP-A in tissues of lung adenocarcinomas using polyclonal and monoclonal antibody. Thirty-three to 55% of tumor tissue of primary lung adenocarcinomas exhibit positive reactions for SP-A staining [28,29,41-43]. No positive reactions are observed in any other histologic type of primary lung carcinomas or in metastatic lung tumors [29] when analyzed by monoclonal antibody PE10. The positive staining is observed not only in the cytoplasm but also in nuclear inclusion bodies of lung adenocarcinomas [29,41], suggesting an abnormal proliferation of nuclear membranes containing SP-A. When well-differentiated and moderately differentiated adenocarcinomas were subtyped by light microscopic findings, Clara cell type and mixed cell type accounted for 33% and 45%, respectively, but type II cell type was merely observed [29]. However, some cancer cells were found to possess osmiophilic lamellar bodies and microvilli characteristic of type II cells by electron microscopic observations [41], suggesting that certain carcinomas originate from type II cells. SP-A and SP-D cannot be markers to determine the cellular origin of lung adenocarcinomas because both proteins are synthesized in Clara cells as well as type II cells. SP-C expression is a potential tool to examine the cellular origin of lung adenocarcinomas, since SP-C is expressed solely in alveolar type II cells [44].

Gazdar et al. [45] examined lung adenocarcinoma cell lines by electron microscopy, immunostaining with anti-SP-A antibody and Northern blots for surfactant proteins. Seven of the 17 adenocarcinoma cell lines exhibited the presence of cytoplasmic structures characteristic of Clara cells or of type II cells. Eight of the nine cell lines expressed SP-A protein and/or mRNA, while a single line expressed SP-C only after dexamethasone induction. A type II cell phenotype may be lost during the establishment of the cell line. The study indicates that SP-A is one of the peripheral airway cell markers and that adenocarcinoma cell lines express peripheral airway cell features. Based on these studies, surfactant gene expression by RT-PCR has been applied to the sensitive detection of metastatic pulmonary adenocarcinomas [46]. SP-A, SP-C, and/or SP-D transcripts were detected in 84.6% of lymph nodes with histologically identifiable metastases of lung adenocarcinomas and in 55.5% of lymph nodes that were negative for metastasis by histological examination. This study indi-
icates that micrometastasis of lymph nodes in lung adenocarcinomas that remains undetectable by conventional microscopic examination can be evaluated by RT-PCR for surfactant proteins.

4. Conclusions and future perspectives

It is now obvious that SP-A and SP-D are specific markers for lung diseases. The measurement of SP-A and SP-D in amniotic fluids and tracheal aspirates evaluates lung maturity and the production of lung surfactant in infants with RDS. The SP-A concentrations in BAL fluids are significantly decreased in patients with ARDS and also in patients at risk to develop ARDS. The prominent increase of these proteins in BAL fluids and sputum is diagnostic for PAP. The concentrations of SP-A and SP-D in BAL fluids from patients with IPF and IPCD are rather lower than those in healthy controls and the SP-A/phospholipid ratio may be a useful marker of survival prediction. SP-A and SP-D appear in the circulation in specific lung diseases. Their serum concentrations significantly increase in patients with PAP, IPF and IPCD. The detection of SP-A and SP-D in sera may be a useful and non-invasive new diagnostic tool for these lung diseases. The successive monitoring of serum levels of SP-A and SP-D may predict disease activity, although they do not correlate with physiological lung functioning tests. SP-A leaks into the circulation in patients with ARDS. It would be also worthwhile to determine the concentrations of serum SP-D in ARDS. SP-A can be used to differentiate lung adenocarcinomas from other types and metastatic cancers, and to detect metastasis of lung adenocarcinomas.

KL-6, a MUC 1 mucin expressed on alveolar type II cells and also on epithelial cells in other organs, has been shown to increase in sera from patients with IPF [47]. Unlike SP-A or SP-D, the KL-6 levels in BAL fluids from IPF patients are also elevated. Future work should include (i) determining how the increases of serum SP-A and SP-D correlate with the pathological changes of interstitial lung diseases, (ii) clarifying the mechanism by which these proteins increase in sera from patients with lung diseases, and (iii) comparing the clinical usefulness among the biomarkers including SP-A, SP-D and KL-6.

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