Expression of Heterogeneous Nuclear Ribonucleoprotein A2/B1 Changes with Critical Stages of Mammalian Lung Development

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Recent reports have demostrated a link between expression of members of the family of heterogeneous nuclear ribonucleoproteins (hnRNPs) and cancer. Overexpression of hnRNP A2/B1 correlated with the eventual development of lung cancer in three different clinical cohorts. We have studied the expression of hnRNP A2/B1 messenger RNA (mRNA) and protein during mammalian development. The expression of hnRNP A2/B1 mRNA and protein are parallel but change dynamically during critical periods in mouse pulmonary development. hnRNP A2/B1 is first detected in the lung in the early pseudoglandular period, peaks at the beginning of the canalicular period, and remains high during the saccular (alveolar) period. In mouse and rat, hnRNP A2/B1 expression is first evident in the earliest lung buds. As lung development progresses, the cuboidal epithelial cells of the distal primitive alveoli show high levels of the ribonucleoprotein, which is almost undetectable in the proximal conducting airways. The expression of hnRNP A2/B1 is restricted in mature lung. Similar dynamic pattern of expression through lung development was also found in rat and human lung. Upregulated expression of hnRNP A2/B1 at critical periods of lung development was comparable to the level of expression found in lung cancers and preneoplastic lesions and is consistent with hnRNP A2/B1 overexpression playing an oncodevelopmental role. **Montuenga, L. M., J. Zhou, I. Avis, M. Vos, A. Martinez, F. Cuttitta, A. M. Treston, M. Sunday, and J. L. Mulshine. 1998. Expression of heterogeneous nuclear ribonucleoprotein A2/B1 changes with critical stages of mammalian lung development. Am. J. Respir. Cell Mol. Biol. 19:554–562.**

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a group of RNA-binding proteins involved in various steps of messenger RNA (mRNA) biogenesis such as splicing and transport to the cytoplasm (1). More than 20 hnRNP proteins ranging from 34 to 120 kD have been described, the most abundant of which are denoted the A, B, and C subtypes. Recent diverse lines of evidence support a relationship between hnRNPs and growth regulation as well as carcinogenesis. For example, mRNA levels of hnRNP A1 and A2 are higher in several immortalized or transformed cell lines than in differentiated human cells (2). The hnRNP A2 has been reported to interact with calmodulin in rat liver cells (3) and, in an *in vitro* system, with the nucleotide sequence of the human telomeric DNA repeat (4). The hnRNP K binds to an upstream region of the c-*myc* gene and has properties of a transcription factor (5, 6). Very recently, the complementary DNA (cDNA) of a proliferation potential protein (P2P-R), a member of the hnRNP A/B family, has been shown to encode a protein domain that binds the tumor supressor protein Rb1, involved in terminal differentiation (7). Members of the hnRNP-C family undergo targeted proteolytic degradation by interleukin-1β–converting enzyme-like proteases during apoptosis (8).

We reported that tumor-associated monoclonal antibody 703D4 can be used as an early detection tool for lung cancer. An immunocytochemical assay of exfoliated bronchial epithelial cells of archival sputum specimens from a high-risk cohort showed 90% accuracy in identifying individuals who would progress to invasive tumor within two years (9). Preliminary analysis of two new trials confirms the utility of this antibody in detecting early lung cancer (10). We recently demonstrated the principal antigen for 703D4 to be hnRNP A2/B1 (11). We also showed that hnRNP A2/B1 mRNA was overexpressed and dysregulated in lung cancer cell lines and a transformed bronchial epithelial cell line when compared with normal primary

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bronchial epithelial cells under conditions of serum starvation (11). The expression of hnRNP A2/B1 in respiratory tissue from individuals without known lung disease was not readily evident (12). In respiratory epithelium evaluated from resected primary lung cancers, high hnRNP A2/B1 expression was found in cells with a range of morphologic appearances from normal to dysplastic to neoplastic (13).

A close relationship exists between mechanisms of oncogenesis and embryonic development, such that many parallels exist between normal development and malignant growth (14–18). Because these independent findings all suggest a relationship between cancer and the hnRNPs, and our previous results point specifically to an association between hnRNP A2/B1 and lung cancer, it is likely that hnRNP A2/B1 may have a significant involvement in lung development. Oncodevelopmental proteins are characterized by being expressed in embryonic development, repressed or downregulated during adult life, and reexpressed during tumor progression (15–18). Some isolated reports do show expression or regulation of hnRNP during development in invertebrates and lower vertebrates: the Xenopus hnRNP A1, A2, and A3 genes are expressed throughout development and in adult tissues (19); and it has been suggested that cell type-specific expression of a particular *Drosophila* hnRNP A/B protein, hrp40, is required for normal development (20).

Because the structure of hnRNP A2/B1 is highly conserved between humans and rodents, and lung development is well studied in rodents, we have evaluated expression of this molecule as both mRNA and protein during stages of mouse development. Lung development in the mouse starts at E9.5 with the outgrowth of paired epithelial buds from the foregut endoderm. In the pseudoglandular stage (E10–E14), epithelial tubes undergo precisely regulated branching morphogenesis to produce proximal conducting airways and distal acinar buds. During the canalicular stage (E14–E16), these buds grow and dilate. The elongated buds become sac-like structures during the saccular stage (E16–P0), a process paralleled by the thinning of the surrounding mesenchyma and the invasion of capillaries, to form a functional air-exchange alveolar compartment. To explore the basic biologic principles that may be involved in the relationship between hnRNP A2/B1 and carcinogenesis, we investigated the expression of this molecule during normal lung fetal development.

Materials and Methods

Rodent Tissues

Sections of Sprague–Dawley rat and NIH Swiss mouse staged embryos (Novagene, Madison, WI) were used. All specimens were fixed for 24 h in 4% buffered paraformaldehyde, embedded in paraffin, and sectioned at 7-μm thickness. For mRNA extraction, E16 and E18 embryos were used. Dams were obtained from Science Applications International Co. (Frederick, MD; protocol 95/125) and killed by CO_2 inhalation. The uterus was quickly removed and kept at 4°C while each embryo was dissected. Tissues were kept at −80°C until extracted.

Human Tissues

Autopsy tissue from human adult and fetal, normal and pathologic lung tissue samples without patient identifying information were obtained under an institution-approved human tissue procurement protocol from the Department of Pathology, University of Navarra, Pamplona, Spain. A total of 32 lung tumor cases were studied, including 12 squamous cell carcinomas, 13 adenocarcinomas, and seven carcinoids. Fetal tissue was from spontaneous miscarriages ranging from 20 to 40 wk in fetal age. Normal human lung was from accident trauma victims thought to be nonsmokers. All the human specimens were fixed in 10% buffered formalin overnight and embedded in paraffin.

Antibodies

The mouse monoclonal antibody (MoAb) 703D4 (IgG_2b_k), was developed and characterized in our laboratory by several molecular techniques that show its specificity for hnRNP A2/B1 (9, 11, 12). The antibody against gastrin-releasing peptide was also previously characterized by our group (MoAb 2A11; IgG_{1k}) (21). The polyclonal antibodies to Clara cell secretory protein (CC-10) and type II pneumocytes (surfactant protein C, SP-C) were a kind gift from Dr. I. Linnoila (National Cancer Institute, Bethesda, MD).

Immunohistochemical Analysis

We used the Histomouse SP kit (Zymed Laboratories Inc., San Francisco, CA) for the immunostaining technique, following manufacturer's protocol with a minor modification. Tissues were incubated with primary antibodies diluted to 10 μg/ml at 4°C overnight and then processed as per kit instructions. All the experiments included a negative control slide in which an isotypic mouse monoclonal control antibody was used. These negative controls did not show any nonspecific tissue reactivity. Omission of second layer and immunostaining with other nonrelated antibodies was also used as a control for the technique. Immunohistochemical expression was scored as previously reported (13).

In Situ Hybridization

An 1145-base pair hnRNP A2 cDNA obtained by reverse transcription–polymerase chain reaction from non-small cell lung cancer (NSCLC) cell line H720 was used to generate the riboprobe. Labeled probes were prepared by using a digoxigenin-RNA labeling kit (Boehringer Mannheim, Indianapolis, IN) and T7 or Sp6 RNA polymerase. Pretreatment of the sections included a Proteinase K digestion step (15 μg/ml for 15 min with adult tissues and 2 μg/ml for 12 min for fetal tissues). Hybridization was performed at 46°C for 20 h in a 20-μl volume containing the probe at 10 ng/μl. After stringency washes, visualization of digoxigenin was performed using a digoxigenin detection kit (Boehringer Mannheim). Sense probe and ribonuclease pretreatment of the sections were used as negative controls.

Isolation of RNA and Northern Blot Analysis

Total RNA was purified by the phenol/chloroform method. A total of 10 μg of total cellular RNA from each tissue was

used for Northern blot analysis. RNA was transferred passively to a Nytran (Schleicher & Schuell, Keene, NH) membrane, hybridized at 68°C for 2 h, and washed. Autoradiography was performed according to standard techniques. Probes used for Northern blot analysis were prepared from a plasmid containing full-length cDNA for hnRNP A2. The hnRNP A1 and K probes were generous gifts of Drs. G. Dreyfuss (University of Pennsylvania Medical School) and D. Levens (Laboratory of Pathology, National Cancer Institute), respectively. Approximately 1×10^6 cpm/ml of probe was used for each Northern blot analysis. Intensity of the bands was estimated using a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA).

Results

Expression of hnRNP A2/B1 in Developing Mammalian Lung

In mammalian lung development, the forming airways expressed hnRNP A2/B1. The highest levels of protein and mRNA expression were found in the epithelial cells of the branching system of tubules of the developing mouse (E10–E16), rat (E12–E18), and human (20 wk onward) lung. Immunocytochemistry showed that hnRNP A2/B1 was present predominantly in the cytoplasm, but was sometimes also present in the nucleus, depending on cell type. In the epithelial cells, cytoplasmic localization was predominant; whereas in fibroblastic and mononuclear infiltrating cells, localization in the nucleus was sometimes seen, especially in human tissues.

Table 1 summarizes the immunocytochemical results obtained in relation to hnRNP A2/B1 expression in mouse and rat lung development. A similar pattern of expression was found in human development. In general, hnRNP A2/B1 can be detected for the first time early in the pseudoglandular period, peaks at the end of the pseudoglandular and the beginning of the canalicular period, and remains high during the saccular (alveolar) period. In the adult lung of all the species studied, the expression of hnRNP A2/B1 is markedly lower than in fetal lung.

Patterns of Expression of hnRNP in Developing Mouse Lung

Immunostaining for hnRNP A2/B1 was demonstrated in the mouse respiratory primordia from E10–E11 onward. At E11 all the specimens showed very light staining in the epithelial cells of the lung buds and in some mesenchymal cells of the forming lung. At E12, two different types of airways were distinguished in relation to hnRNP A2/B1 expression. The more proximal tubules that develop into conducting airways, lined by a simple columnar epithelium, were negative for hnRNP A2/B1; at the same time, the distal undifferentiated cuboidal epithelium lining acinar tubules moderately expressed hnRNP (data not shown). Around Day 14, a marked increase in the mRNA (Figure 1A) and protein levels (Figures 1B and 1C) of hnRNP A2/B1 was observed in the distal acinar tubules, whereas expression in the proximal columnar epithelium of the future conducting airways remained negative or very low (Figure 1C). On E16, during the late canalicular phase of lung development, localization of hnRNP A2/B1 mRNA (Figure 1D) and protein (Figures 1E and 1F) was similar to that during the pseudoglandular stage. The low cuboidal epithelial cells characteristic of the sac- or pouch-like structures, resulting from the expansion and differentiation of the cuboidal epithelial acinar tubules, also expressed hnRNP (Figure 1F). Expression of hnRNP mRNA was similar to the pattern of immunostaining (compare Figures 1A and 1B, 1D and 1E). On E14 and E16, levels of hnRNP A2/B1 in the mesenchymal cells of the developing lung were either negative or very low. The appropriate controls were all negative, including *in situ* hybridization using a sense probe (Figure 1G) and immunostaining using an isotypic control antibody (Figure 1H). In alveoli of adult mouse lung, there was no hnRNP A2/B1 protein detectable using the same concentration of the primary antibody as used

TABLE 1

Immunocytochemical detection of hnRNP A2/B1 in lung development

Subject, Developmental Stage	Bronchial Bud	Proximal Epithelium	Distal Epithelium
Mouse			
E10	4	–	–
E12	–	0	4
E14	–	0	6
E15	–	2	6
Mature	–	0	0
Rat			
E14	–	0	4
E16	–	2	6
E18	–	2	4
Mature	–	0	0
Human			
Fetal (20–28 wk)	–	3	5
Perinatal	–	2	2
Adult*	–	0/2	0/2

– indicates structure not present at this stage. Values in the table are the sum of a score for intensity (from 0 = negative to 3 = very intense) and a score for extension (from 0 = 0% to 3 = 100% of the cells).

* Normal bronchial epithelium without observable immunoreactivity, except for focal inflammatory areas where score for immunoreactivity was 2.

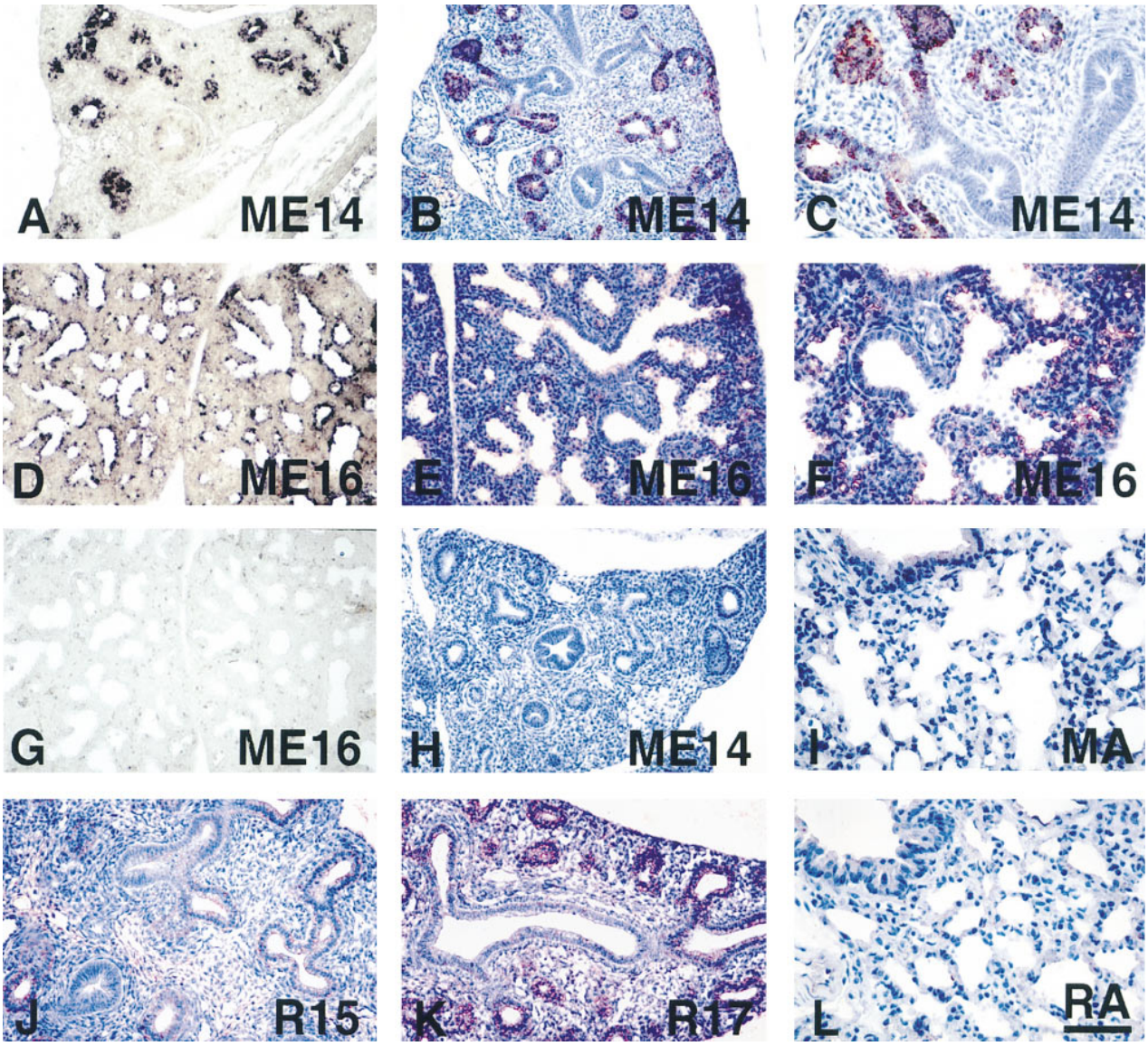

Figure 1. Expression of hnRNP A2/B1 in the developing lung of mouse (*A–I*) and rat (*J–L*) using *in situ* hybridization (*A*, *D*, *G*) or immunohistochemistry with the monoclonal antibody 703D4 (*B*, *C*, *E*, *F*, *I–L*). In both species, stronger levels of hnRNP A2/B1 expression can be detected in the airway distal epithelium than in the forming central bronchi (*see* especially *C*, *I*, and *K*). ME14 and ME16: Mouse embryo of 14 and 16 d; RE15 and RE17: rat embryo of 15 and 17 d. Expression of hnRNP A2/B1 in adult mouse (MA; *I*) or rat (RA; *L*) is greatly reduced compared with that of embryonic lung processed in the same conditions. *I* is a section of adult mouse immunostained for hnRNP A2/B1 in the same conditions as (*B*) or (*E*). (*G*) is a sense control for *in situ* hybridization in a consecutive section of (*D*). (*H*) Section of 14-d mouse embryo equivalent to (*B*) immunostained using monoclonal antibody isotypic to 703D4 as primary antiserum. (*Scale bar* shown in *L* corresponds to 70 μm in *C*, *F*, *I*, and *L*; in panels *A*, *B*, *D*, *E*, *G*, *H*, *J*, and *K* it corresponds to 130 μm.)

with the embryonal tissues (Figure 1I). The bronchiolar epithelium of the adult mouse was also either negative or very weakly positive for hnRNP A2/B1.

Patterns of hnRNP Expression in Developing Rat Lung

The patterns of expression in developing rat lung were also analyzed (Figures 1J–1L). The localization of hnRNP A2/B1 and its mRNA paralleled that found for the mouse, in particular the early establishment of two patterns of expression (low in proximal versus high in distal airways) and the marked increase of hnRNP expression in the developing distal airways at the end of the canalicular period (E17–E18 in the rat). Slightly higher expression of hnRNP A2/B1 in the developing central airways and in some of the mesenchymal cells of rat lung appeared to be the only difference between the two species.

hnRNP Expression in Developing Human Lung

Similar to the findings for mouse and rat, hnRNP A2/B1 expression was found in the epithelial cells of the acinar

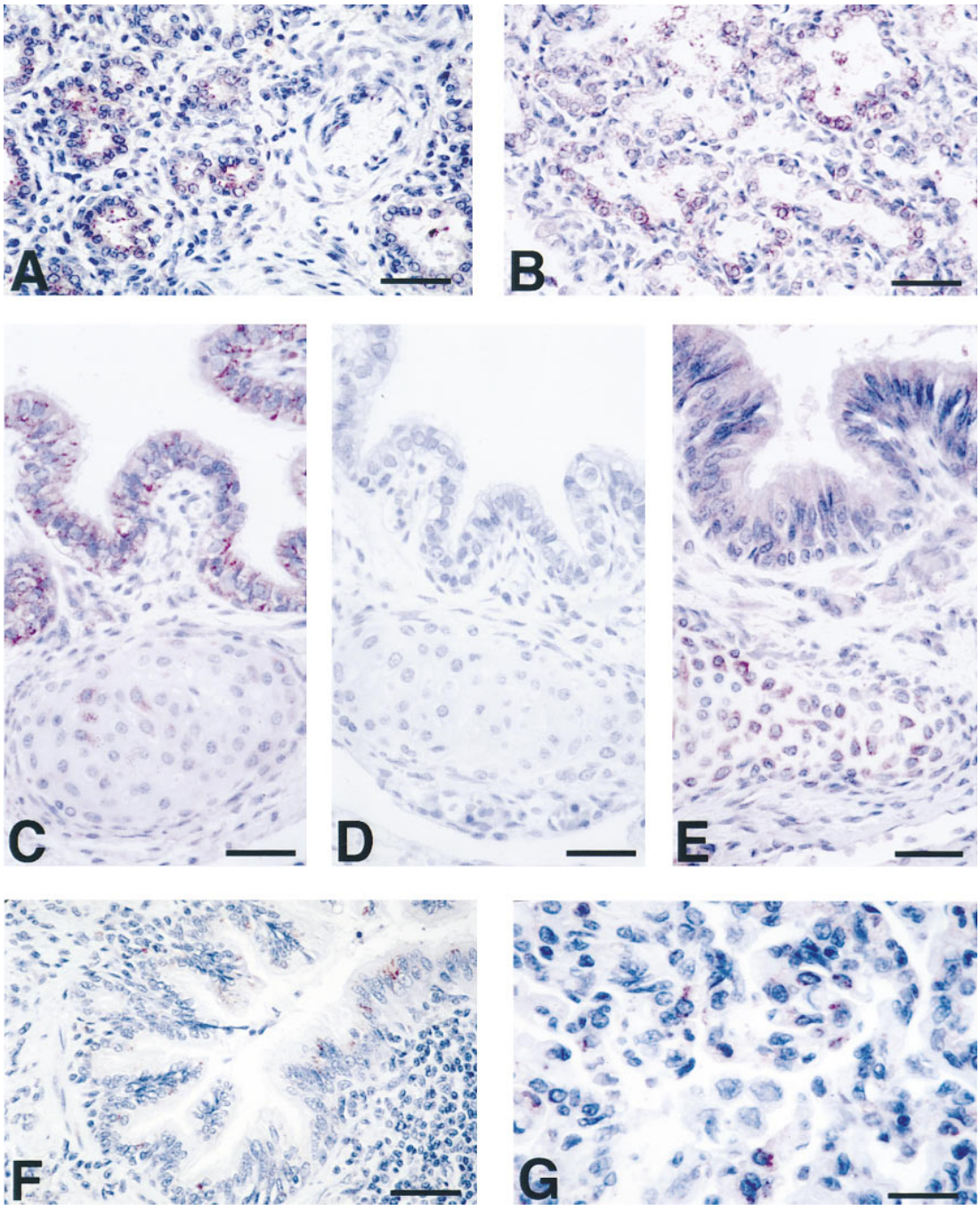

Figure 2. Immunocytochemical detection of hnRNP in different stages of developing human lung. (*A*) Lung parenchyma at 20 wk; (*B*) lung parenchyma at 28 wk; (*C*) bronchus circa 25 wk; (*D*) serial section of (*C*), to which the isotypic control monoclonal antibody was applied; (*E*) bronchus at 28 wk. Note that forming chondrocytes also express hnRNP A2/B1. (*F*) Bronchiolus of a newborn. (*G*) Lung parenchyma of a newborn. (*Scale bar* in *A–F* is 70 μm; in *G* is 40 μm.)

tubules of 20-wk gestation human fetal lung (Figure 2A), which corresponds to the mid-canalicular stage of lung development. Consistent with our observations in rodent lung, human 28-wk gestation fetal lung (late canalicular to early saccular period) demonstrated high levels of hnRNP A2/B1 (Figure 2B) with the epithelial cells of primitive alveoli strongly expressing hnRNP A2/B1. The sole exception to the mouse and rat pattern is in the developing intrapulmonary bronchi and bronchioles of midgestational human fetal lung which expressed hnRNP A2/B1. In particular, immunoreactivity could be observed in the prospective bronchial/bronchiolar epithelium of early (20-wk) human fetal lung, at least in some of the epithelial cells. These hnRNP A2/B1-positive cells were not Clara cells or pulmonary neuroendocrine cells as determined by immunostaining of serial tissue sections for the Clara cell secre-

tory protein (CC10) or for bombesin-like neuropeptide (data not shown). In later stages of development (28 wk), expression of hnRNP A2/B1 in the human bronchial and bronchiolar epithelium increased markedly (Figures 2C–2E).

In the newborn lung, expression was low but still detectable (Figures 2F and 2G) both in bronchial epithelium and type II pneumocytes. In some bronchial epithelial columnar cells, supranuclear granular staining was observed. In general, the expression of the hnRNP A2/B1 gene in the alveolar compartment of normal human adult lung was low and usually undetectable using either immunocytochemistry (Figure 3A1) or *in situ* hybridization (Figure 3A3) detection systems. Type II pneumocytes were usually negative (Figures 3A1 and 3A2). The expression in adult bronchial epithelium was also low in most cases, but was higher in the epithelial cells of some airways present at uninvolved "normal" areas adjacent to tumors. In these cases the supranuclear granular staining pattern was readily observed. Smooth muscle cells of the blood vessels and chondrocytes of the larger airways occasionally stained with 703D4 (data not shown).

Expression of hnRNP in Hyperplastic and Neoplastic Lung

We previously reported hnRNP A2/B1 expression in "field carcinogenesis" using non-neoplastic tissues surrounding resected primary lung tumors (13). In the present study we used *in situ* localization of hnRNP A2/B1 mRNA to con-

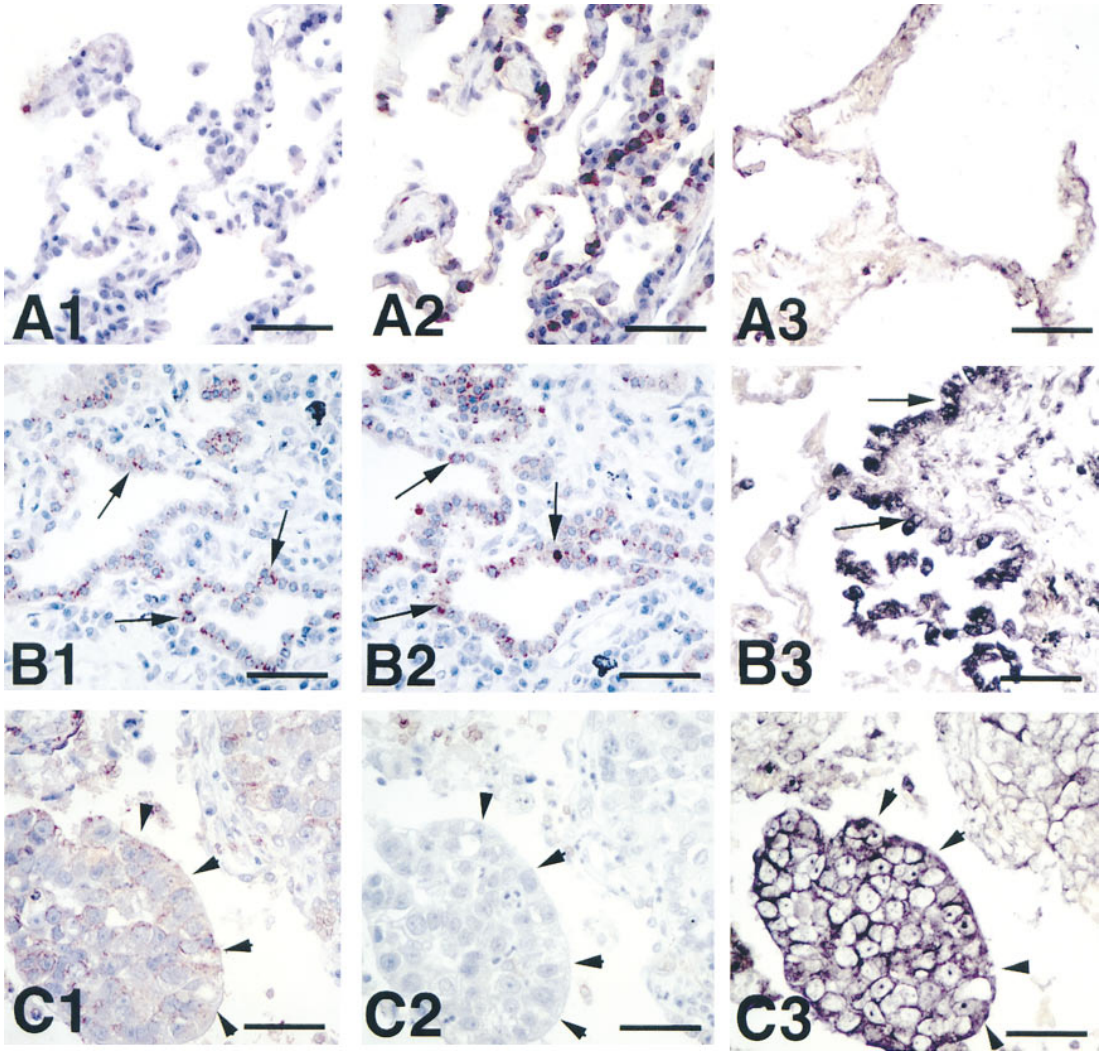

Figure 3. (*A*) Normal human adult lung. *A1:* immunostained for hnRNP A2/B1; the lung parenchyma expresses very low, almost undetectable, hnRNP A2/B1. *A2:* The same area immunostained with an antibody against SP-C to show the abundant type II pneumocyte population. *A3: In situ* hybridization for hnRNP A2/B1 in normal lung parenchyma, showing low levels of mRNA. (*B*) Type II cell hyperplasia. *B1:* Immunostained for hnRNP A2/B1 showing high expression in the hyperplastic type II cells (*arrows*). *B2:* Serial section immunostained with an antibody against SP-C. Hyperplastic type II cells (*arrows*) are strongly stained. *B3: In situ* hybridization for hnRNP A2/B1 in a similar region, showing high mRNA expression in the hyperplastic type II pneumocytes (*arrows*). (*C*) Lung adenocarcinoma. *C1:* Immunostained for hnRNP A2/B1 showing high expression in the tumor cells (*arrowheads* point to a group of tumoral cells). *C2:* The same area using an isotypic monoclonal antibody as negative control. *C3: In situ* hybridization for hnRNP A2/B1 in a serial section, showing high mRNA expression in the tumor cells. (*Scale bar* in all figures represents 70 μm.)

firm those previous findings, using previously unanalyzed cancer cases. High expression of hnRNP was demonstrated in a variety of NSCLC both at the protein and mRNA levels (Figure 3C1–3C3). Parallel immunocytochemistry and *in situ* hybridization was carried out in 22 lung cancer specimens. In 20 (92%) of the cases, both protein and mRNA for hnRNP A2/B1 were detected; 75% of these double-positive cases showed comparable expressions of protein and mRNA according to the relative criteria of intensity and extension that we used. Type II cell hyperplasia also showed strong expression of hnRNP A2/B1 both by immunocytochemical and *in situ* hybridization techniques (Figures 3B1–3B3). In these hyperplastic cells, the hnRNP A2/B1 protein seems to be concentrated in granular-like cytoplasmic structures, but nuclear staining was also observed in some tumor cells of specimens independent of histology. No apparent correlation between presence of nuclear staining and tumor type was found. The levels of intensity of immunostaining of the tumoral cells were comparable to, and in some cases higher than, those observed in developing lung epithelial cells.

Northern Blot Analysis of hnRNP Gene Expression during Rat Development

Densitometric analysis of Northern blots of fetal, neonatal, and adult rat lung mRNAs (Figure 4) showed that the levels of hnRNP A2/B1 transcripts are significantly higher during late fetal stages than in the adult stage. The intensity of the band for hnRNP A2 mRNA from E16, E18, P1, and adult rats diminished with maturation. Decreased hnRNP A2/B1 mRNA expression was consistently observed in three analyses of replicate samples. Between E16 and adult, lung hnRNP A2/B1 mRNA expression was decreased an average of 4.9-fold. Similar decreases in the levels of mRNA could be detected when using probes specific for hnRNP A1 and hnRNP K.

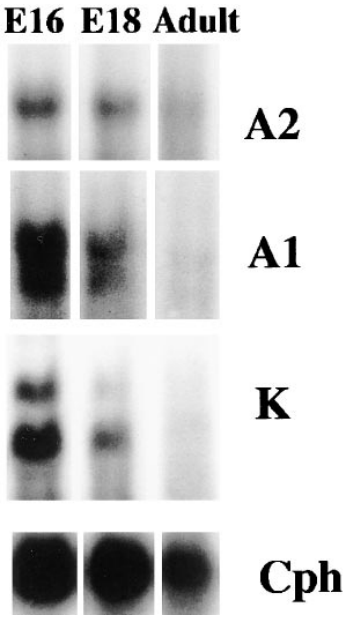

Figure 4. RNA blot analysis of different hnRNPs during rat lung development. The housekeeping gene cyclophilin (Cph) was blotted at the same time to show differences in RNA loading.

Discussion

The present study shows for the first time the expression of hnRNPs during mammalian development. This data is consistent with our hypothesis of hnRNP regulation playing a role in lung growth and differentiation and its dysregulation being involved in the process of carcinogenesis. We evaluated the full course of fetal lung development in rodents and found hnRNP A2/B1 expression first evident in the central regions of the developing lung, with a gradual migration of expression to the periphery of the lung with a timing that parallels the time course for the maturation of the developing rodent lung. The regulated expression pattern of hnRNP A2/B1 during fetal development, downregulation in normal adult tissues, and reexpression in cancer is consistent with it being an oncofetal molecule. Our previous report demonstrates that hnRNP A2/B1 expression is increased in dysregulated cell populations (11). Developing mouse and rat lung show increased expression of hnRNP A2/B1 in actively proliferating immature cells. The hnRNP A2/B1 can be detected for the first time in the respiratory system in the undifferentiated columnar epithelium of the branching tubular system of the lung primordium. In the pseudoglandular stage, when the primordial lung buds differentiate into the prospective bronchial system and the prospective respiratory system (22), detection of hnRNP A2/B1 is restricted to the immature epithelial cubic cells of the more distal (prospective respiratory) airways. At the same stage, the more differentiated cells of the central bronchial system do not express detectable levels of hnRNP A2/B1 protein or mRNA. Expression of hnRNP A2/B1 in the maturing alveolar system is also high during the later canalicular period of lung development, remaining low or undetectable in the columnar epithelium of the prospective bronchioli. This dynamic pattern of expression is consistent with tight regulation of this molecule in fetal development. A similar lung distribution has been shown for the oncofetal carbohydrate antigen Le^x, that is a mediator of cell–cell interactions (17). A gradient of expression (higher in epithelium of forming central airways to lower in distal pre-respiratory ducts) has also been reported for Bcl-2 in fetal mouse lung (23). Bcl-2 has been proposed to act as a survival factor during morphogenesis by preventing entry of the cell into the apoptotic pathway.

The pattern of expression of hnRNP A2/B1 in the developing lung was consistent in the mouse, rat, and human specimens studied. The only exception was in the epithelium of the developing human intrapulmonary bronchi and bronchioles, which show expression of hnRNP A2/B1 in a subset of epithelial cells during the earlier stages of fetal development studied (20 wk) and increasing expression of hnRNP in most of the bronchial epithelial cells during the perinatal period. The higher expression of hnRNP A2/B1 in human airway epithelial cells in the late fetal and early postnatal period might be related to ongoing maturation because the airway mucosa of the newborn human, in contrast to rodent lung, is not yet fully differentiated (24). In this study, we observed a sharp rise of hnRNP A2/B1 expression in the type II precursor cells of the embryonic mouse lung around E14. The type II precursor cell has received considerable interest because it shows common antigenic and ultrastructural features with lung cancer cells

(25). In rat and human fetal lung, hnRNP expression is also high in the equivalent cell type of the epithelium of the distal airways at the same developmental period (pseudoglandular stage). The cubic epithelial cell of the forming distal airways of the rodent and human lung is considered to be the common progenitor cell for type I and type II pneumocytes (22). During the early stages of mouse lung development (E13–E15) distal epithelial cells co-express markers for multiple differentiated lung epithelial cell types, including Clara cells and neuroendocrine cells (26). Many types of lung tumors also express a variety of neuroendocrine and non-neuroendocrine marker antigens. Interestingly, the hyperplastic type II cells found in areas of adult human lung adjacent to tumors consistently show very high levels of hnRNP A2/B1. Type II cell hyperplasia has been postulated as a preneoplastic lesion for NSCLC. We have previously shown increased expression of the enzyme responsible for post-translational activation of autocrine peptide hormones in type II hyperplastic cells (27); and a subset of type II cell hyperplasia has been shown to have increased p53 immunostaining, consistent with p53 mutation (28), and shows K-ras oncogene activation (29).

Immunolocalization of hnRNP A2/B1 in the cytoplasm of many cell types is consistent with the reported function of this ribonucleoprotein in mRNA shuttling between the nucleus and the cytosol (30). Although most of the hnRNP proteins have been immunolocalized to the nucleus, cytoplasmic expression of several types of hnRNP proteins has also been reported in several tissues (31). Accumulation of the ligand, either in the nucleus or the cytoplasm, may account for the detection using the reported immunocytochemical method. Conversely, conformational changes may result in increased recognition of hnRNP A2/B1 in the cytoplasm. In either case, the pattern of granular staining in several epithelial cells suggesting cytoplasmic hnRNP accumulation merits further investigation.

Several authors have reported an association of hnRNP expression with actively proliferating cells (2, 11, 32). Biamonti and colleagues (2) have previously shown the expression of hnRNP A1 in a human teratocarcinoma cell line (NT2/D1) and proposed an upregulation of this protein in highly proliferative cell populations. These authors also showed high levels of hnRNP expression in transformed cells, which is consistent with our data for hnRNP A2/B1 in lung tumor cells (11, 13). Preliminary analysis suggests that the elevation of hnRNP A2/B1 mRNA in the developing lung may be part of a general increased expression of the members of the hnRNP family. The hnRNP A2/B1, A1, and K show increased expression during development, supporting this hypothesis and pointing toward a general regulation of the mRNA processing machinery. An increase in the levels of multiple hnRNP proteins might be a requirement to sustain the higher activity of replication, transcription, and translation that occurs during proliferation and/or differentiation. Certainly regulation of splicing dynamics could differentiate a mature from a fetal phenotype and, by analogy, a normal from a malignant phenotype. To understand the role of hnRNPs in cancer or development, further discrimination is needed between the role of hnRNP family members in defined contexts, such as with spliceosomes, and specific functions of particular hnRNP molecules as transcription factors (6, 33), cell cycle modulated nuclear protein kinase substrates (34), Rb-1 binding proteins (7), and non-terminal differentiation markers (7).

We report here for the first time changes in the levels of hnRNPs during development, in particular an upregulation of hnRNP A2/B1 during the development of the mammalian respiratory system, that parallels the overexpression of this ribonucleoprotein during lung carcinogenesis. The pattern of expression for hnRNP A2/B1 for rat, mouse, and human lung development is consistent at both the protein and the message level. Moreover, in human carcinogenesis, the pattern of hnRNP A2/B1 expression recapitulates fetal lung development with expression especially in the case of type II cell hyperplasia. Our data are consistent with the hypothesis that hnRNP A2/B1 is an oncodevelopmental protein. This correlative report suggests that further functional studies, such as with transgenic knockout mice, should be justified to definitely evaluate the role of hnRNP A2/B1 in fetal lung development.

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