A Novel Ribonucleoprotein Complex Defined by Monoclonal Antibodies to NIH 3T3 Cells Transfected with Human Pancreatic Adenocarcinoma DNA

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ABSTRACT

Three monoclonal antibodies elicited to NIH 3T3 cells transfected with DNA from a human pancreatic adenocarcinoma cell line recognized a novel ribonucleoprotein complex. Minimally, this ribonucleoprotein complex contained a M, 240,000 protein (by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and two RNA species with apparent sizes of 1.5 and 3.0 kilobases (by formaldehyde agarose gel electrophoresis). In addition to a cytoplasmic and nuclear subcellular localization, the RNA antigen was secreted from human tumor cell lines and NIH 3T3 cells transfected with pancreatic tumor DNA (inhibitable by monensin) and was apparently not a viral or Mycoplasma contaminant. The ribonucleoprotein antigen was detected in some normal tissues by immunoperoxidase but was not found in or secreted from in vitro cultured normal human fibroblasts, nontransfected or spontaneously transformed NIH 3T3 cells, or normal peripheral blood leukocytes.

INTRODUCTION

In an effort to define novel tumor-associated antigens, we previously described 10 murine monoclonal antibodies which were elicited to antigens expressed on NIH 3T3 cells that were transformed by transfection with high molecular weight DNA from a human pancreatic adenocarcinoma cell line (HPAF) (1). These antibodies were originally selected for their reactivity with HPAF cells and transfected NIH 3T3 cells and lack of reactivity with nontransfected NIH 3T3 cells. As such, they define either human antigens expressed in the transfected NIH 3T3 cells or mouse antigens that were induced in the NIH 3T3 cells by the transfection and transformation and are immunologically cross-reactive with a human counterpart. A number of these antibodies were shown to be reactive with intracellular antigens on human pancreatic adenocarcinoma cell lines, in contrast to our previous studies which emphasized antibodies to cell surface determinants on the immunizing human tumor cells (2). Three of the antitransfectant antibodies (9F9, 13B8, and S1D11), which were produced by hybridomas made with spleen cells from three different mice, demonstrated a similar nuclear and cytoplasmatic pattern of reactivity by immunofluorescence testing with a number of human pancreatic cell lines. This report presents evidence that the antigen recognized by these three antibodies was a novel RNP. Minimally, the RNP contained a M, 240,000 protein and two RNA species with apparent sizes of 1.5 and 3.0 kilobases, which distinguished it from most previously reported RNPs. In addition to the cytoplasmic and nuclear localization, the antigen was secreted from human tumor cell lines and NIH 3T3 cells transfected with HPAF DNA and was apparently not a viral or Mycoplasma contaminant. The antigen was found in some normal tissues by immunoperoxidase but was not found in or secreted from in vitro cultured normal human fibroblasts, nontransfected or spontaneously transformed NIH 3T3 cells, or normal peripheral blood leukocytes.

MATERIALS AND METHODS

Cell Lines and Monoclonal Antibodies. The HPAF cell line, NIH 3T3 cells, and transfected NIH 3T3 cells have been previously described (1). The primary NIH 3T3 cell line transformed by transfection with DNA from the T-24 bladder carcinoma (T-24) was kindly provided by Dr. Stuart Aaronson, NIH. The generation and selection of monoclonal antibodies 9F9, 13B8, and S1D11 have been previously described (1). Other cell lines were obtained from: American Type Culture Collection (Capan 1, Capan 2, Mia PaCa, DU 145, Colo 320, K562, HL-60, U937, SK Mel 28, and GM3498B); Jeff Dawson, Duke University (Molt 4, HSBI, CEM, Daudi, and REH); Hilliard Seigler, Duke University (DU-Mel); and Holger Kalthoff, University of Hamburg (QGP-1 and RWP-2).

Immunoprecipitation. The S1D11 (IgG1) and 9F9 (IgG2b) antibodies used in these studies were affinity purified from mouse ascites on Protein A-Sepharose columns (3), concentrated by ammonium sulfate precipitation (40%), dialyzed against PBS, and resuspended at approximately 10 mg/ml in PBS. Antibody 13B8 (IgM) was purified from mouse ascites by taking the excluded volume from Sephadex G-200 columns and dialyzing these against deionized distilled water (causing the IgM antibody to precipitate). IgM precipitates were dissolved in PBS at 10 mg/ml. Immunoprecipitations were performed using 10 µl of human serum or 10 µl of purified monoclonal antibodies and a goat anti-mouse immunoglobulin second antibody, at an optimal concentration determined by a quantitative equivalence titration for precipitation, or Pansorbin (Calbiochem). Immunoprecipitations were always performed with equivalent amounts of similarly purified isotype-matched control antibodies, which yielded precipitated pellets of equal size to the test antibodies. Secondary antibody immunoprecipitations were performed at 5°C for 12–18 h, followed by: three washes in 525 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 0.02% NaN3, 1% Nonidet P-40; two washes in 525 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 0.02% NaN3, 0.05% Nonidet P-40; and, finally, three washes in 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.5), 0.02% NaN3. Pansorbin immunoprecipitations were performed using Pansorbin prewashed 10 times in NET 2T buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.4, 0.5% sodium deoxycholate, 0.5% Nonidet P-40, 0.1% SDS). Human sera or murine monoclonal antibodies were incubated with radiolabeled cell lysates or supernatants for 30 min at 4°C, and then 10 µl of washed Pansorbin were added for 30 min at 4°C. The immunoprecipitates were then washed 5 times in NET 2T plus 2% bovine serum albumin.

Radiolabeling. Metabolic labeling of cell proteins was accomplished by starving the cells in the appropriate deficient medium (GIBCO Selectamine or a custom-made phosphate-free medium) for 1 h before adding the indicated radiolabel for a 4–6-h period. When labeling with [35S]cysteine, [3H]methionine, or [3H]glucine, we typically used 1 µCi of label/5 × 106 cells. Two µCi/5 × 106 cells of [3P]orthophosphate or [3H]uridine was used. We employed a minimum of 106 trichloroacetic acid-precipitable counts from each cell fraction for each immunoprecipitation.

Cell Fractions. Immunoprecipitations were performed on spent supernatants from cultured cells, cytosolic lysates, and nuclear lysates. Supernatants were cleared of cell debris by centrifugation (10,000 g, 5 min) and used directly for immunoprecipitation or antigen purification. Initially, experiments were performed on cells cultured in 10% fetal bovine serum. Once it became evident that the RNP antigen was...
secreted from cells, we adapted HPAF cells for culture in protein-free Iscove's modified Dulbecco's modified Eagle's medium (GIBCO) with penicillin, streptomycin, and glutamine as the only supplements. This material, which was free of fetal bovine serum or other protein contaminants, served as the antigen source for RNA and protein purification and characterization. Cytosolic lysates were prepared by dissolving the cells in 10 mM Tris-HCl (pH 7.2), 0.15 mM NaCl, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride. Nuclei were lysed in 10 mM Tris-HCl (pH 7.2), 0.15 mM NaCl, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and the DNA were sheared by vortexing.

Guaniidine CsCl Density Gradients. Two types of CsCl density gradients were utilized. For total cellular RNA preparation, the previously described CsCl cushion technique was employed (1). For separation of radiolabeled RNA from radiolabeled proteins for subsequent immunoprecipitation, an equilibrium density gradient with guaniidine-HCl was employed. This procedure, based on the method of Carlstedt et al. (4), involved loading either immunoprecipitated material or radiolabeled HPAF spent medium with 40% (w/v) CsCl plus 4 M guanidine HCl at a final density of 1.42 g/ml into tubes of a Beckman SW 41 Ti rotor. The gradients were formed by centrifugation at 28,000 rpm in a Beckman L-2 ultracentrifuge at 10°C for 96 h. Gradient fractions were dialyzed against PBS and either counted or immunoprecipitated, as indicated in the text. Densities were determined by weighing 100 μl.

SDS-PAGE and Agarose Gel Electrophoresis. SDS-PAGE was performed as previously described (3, 5). Agarose gel electrophoresis was carried out using the indicated percentage of agarose in 0.1 mM Trisphosphate buffer (pH 8.0) with 0.01% EDTA and 0.1% SDS. For some experiments and Northern blots, formaldehyde-agarose gels were used. These contained 40 mM morpholinopropansulfonic acid, 10 mM sodium acetate, 1 mM EDTA, and 2200 mM formaldehyde. Gels were typically electrophoresed for 18 h at 25 V. Gels were silver stained as previously described (6).

Immunofluorescence and Immunoperoxidase. The techniques for the indirect immunofluorescence and the immunoperoxidase techniques were as previously described (2). Cell permeabilization was accomplished by first fixing the cells in 2% parafomaldehyde in Ca- and Mg-free PBS, followed by permeabilization in PBS with 0.01% saponin and 0.25% gelatin.

RNA Purification and Northern Blots. For some experiments, RNA was purified from immunoprecipitates by dissolving the precipitates in 0.5% SDS, 250 μg/ml proteinase K, 1 unit/μl RNasin (Promega), 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, for 30 min at 50°C, followed by two extractions with Tris-equilibrated phenol and three extractions with chloroform/isooamyl alcohol (24/1). The RNA was then precipitated by adding 2.5 volumes of ethanol and was stored as a precipitate under ethanol until use. Northern blots were performed on RNA separated by agarose gel electrophoresis, using standard procedures (7). The ribosomal DNA probe (pDmra51#l) used in these studies was kindly provided by Dr. Sharyn Endow, Duke University Medical Center (8).

RESULTS

Subcellular Localization and Tissue Distribution of the S1D11 Antigen. MAb S1D11, 9F9, and 13B8 were previously observed to have a similar intracellular localization among different transformed human cell lines (1). Diffuse cytoplasmic and focal nuclear localization in HPAF cells was noted by indirect immunofluorescence of permeabilized cells (Fig. 1A).

A survey of several normal tissues for expression of the antigen defined by these antibodies, using an immunoperoxidase technique, revealed its presence in some (pancreas, colon, stomach) but not all (ileum, jejunum, esophagus, breast, kidney, spleen, tonsil, liver) normal tissues. Tumors of the breast, colon, and pancreas also expressed the antigen. However, as will be described later, we have been able to detect the antigen by immunoprecipitation in a number of human cell lines which were judged negative for antigen expression by immunoperoxidase or immunofluorescence techniques. It is, therefore, probable that the actual tissue distribution would be more extensive if detection techniques more sensitive than immunoperoxidase were available for tissue sections.

Molecular Characterization of the S1D11 Antigen. Initial attempts to define the molecular characteristics of this antigen included immunoprecipitation of different radiolabeled lysates and supernatants from HPAF cells. In these experiments, the three antibodies immunoprecipitated antigens with apparently identical molecular properties that included two different molecular components: several molecules which could be labeled with [35S]cysteine and [3H]methionine or [3H]leucine and a larger species that could be labeled with [32P]P, which would not run through a 4% stacking gel in SDS-PAGE. The 32P-labeled precipitates were further resolved into two bands by electrophoresis in 1.5% agarose gels containing 0.1% SDS. None of the bands were labeled with [3H]glucosamine. This raised the possibility that the larger species was a phosphoprotein, so we conducted a phosphoamino acid analysis (9) on electrophoresed material from gel slices containing this band. However, no phosphorylated amino acids were detected. Since a number of other molecular species can be labeled with [32P]P, including DNA, RNA, phospholipids, and ADP-ribosylated proteins, we tested 32P-labeled immunoprecipitates for the presence of these moieties by denaturing them in 0.1% SDS and digesting them with DNase, RNase, and proteinase K. The 32P-labeled material was degraded by RNase and was unaffected by DNase or proteinase K (Fig. 2). The presence of the RNA moiety was confirmed when immunoprecipitates of [3H]uridine-labeled material revealed two bands with identical properties of migration in agarose gels (Fig. 3). Size estimation (compared to 18S and 28S rRNA) for the two RNA bands based on their migration in formaldehyde-agarose gels indicated that they were 1.5 and 3.0 kilobases in length. Pretreatment of supernatants and lysates with RNase before immunoprecipitation did not affect the RNA moiety, suggesting that bound protein may protect the RNA from degradation.

Initial immunoprecipitates of [35S]cysteine- and [3H]methionine-labeled cell lysates that were washed in high salt buffers (525 mM NaCl) contained only a M, 240,000 molecule. Subsequent immunoprecipitations utilizing wash buffers with lower salt concentrations (150 mM NaCl) precipitated additional molecules with apparent molecular weights around 110,000 and 45,000. Taken together, the radiolabeling and immunoprecipitation data suggested that this antigen was a ribonucleoprotein complex.

Data presented in Fig. 4A compare immunoprecipitation of HPAF cell lysates with MAbs SID11 and 9F9 to immunoprecipitations with sera from autoimmune disease patients with known antibody specificities for Sm, La, Ro, and ribosomal RNA, and high-salt nuclear fractions of HPAF cell nuclei (kindly provided by Dr. Jack Keene, Duke University Medical Center). In the course of characterizing this antigen, we were surprised to find detectable amounts of the RNP complex in spent medium from cultured HPAF cells. Fig. 4B, C, and D presents immunoprecipitations of HPAF supernatant with the same antibodies as those depicted in Fig. 4A. The data presented in Fig. 4 reveal that MAbs 9F9 and S1D11 immunoprecipitate protein bands of molecular weights 240,000, 110,000, and 45,000 which were not seen in control lanes of normal serum, irrelevant MAb isotype controls, or a variety of autoimmune sera that immunoprecipitated a M, 60,000 Ro molecule, M, 50,000 La molecule, M, 35,000 and 31,000 rRNP molecules. Sm molecules were not resolved in SDS-PAGE gels of the percentage (7.5%) used here. The M, 31,000
and 35,000 rRNP molecules and the $M$, 50,000 La molecule could also be labeled with $^{32}$P, and immunoprecipitated from cell lysates, whereas the $M$, 45,000 molecule precipitated by MAbS 9F9 and S1D11 did not label with $^{32}$P (data not shown). The autoimmune sera and control antibodies did not specifically immunoprecipitate any antigens from supernatants above HPAF cells labeled with $^{32}$P or $^{35}$S, whereas MAb S1D11 specifically immunoprecipitated the $M$, 240,000, 110,000 and 45,000 molecules from $^{35}$S-labeled supernatants (Fig. 4B) and immunoprecipitated only the very large moiety from $^{32}$P-labeled supernatants, which would not enter the SDS-PAGE gel (Fig. 4C) and which was eliminated upon RNase treatment (data not shown).

Secretion of RNP Antigen from Cell Lines. The fact that the RNP complex was detected in spent medium from cultured HPAF cells raised the possibility that the antigen was actually a Mycoplasma or a virus. Although Mycoplasma screening of all cell lines was a routine part of the laboratory regimen, we retested the HPAF cells for Mycoplasma using both Hoechst DNA staining technique and a commercially available in vitro diagnostic test for cell cultures (Mycotrim; New England Nuclear). The results showed that the HPAF and other cell lines expressing the RNP were negative for Mycoplasma. In addition, supernatants from HPAF cells were incubated with antigen-negative NIH 3T3 cells in an effort to convert them to an antigen-positive state. We also monitored the exposed NIH 3T3 cells for morphological changes. After 4 weeks of culture, the exposed NIH 3T3 cells remained negative for antigen by immunoprecipitation and showed no morphological evidence of transformation. HPAF cells were also negative for reverse transcriptase activity. Finally, electron microscopy of negatively stained sections of S1D11 immunoprecipitates showed no
RIBONUCLEOPROTEIN COMPLEX

These findings suggested that the RNP antigen in the supernatant, therefore, either was actively secreted or was a byproduct of dying cells. Because the cell viability always remained high (greater than 95%) during the 4–6-h radiolabeling period used to generate RNP antigen preparations, we felt that cell death was not the explanation. Further support for the hypothesized active secretion was obtained when it was found that the calcium ionophore monensin inhibited the appearance of immunoprecipitable RNP in HPAF spent medium (Fig. 5) but did not affect its accumulation in the cytosol. Because the spent supernatant form of the antigen represented a potentially cleaner source for purification then cell lysates, we adapted the HPAF cell line to growth in serum-free, protein-free, unsupplemented Iscove’s medium (GIBCO) and used spent supernatants from these cells for subsequent purification and characterization studies described later.

Expression of the RNA Antigen in NIH 3T3 Cells Transformed by Transfection with HPAF DNA and in Other Human Cell Lines. We have performed immunoprecipitations to screen a number of HPAF-transfected NIH 3T3 cells, normal NIH 3T3, and NIH 3T3 cells transformed by the c-H-ras oncogene of the EJ bladder carcinoma cell line for the presence of these antigens. Examples of these experiments are shown in Figs. 6 and 7 for the protein and RNA moieties, respectively. These data show that proteins (M, 240,000, 110,000, and 45,000) and RNA (1.5 and 3.0 kilobases) molecules of similar size to those seen in HPAF can be immunoprecipitated from the NIH 3T3 cells transfected with HPAF DNA. The lysates from the transfected NIH 3T3 cells also yielded, upon immunoprecipitation, several other bands with molecular weights of 40,000, 58,000, and 75,000 (Fig. 6), which showed SDS-PAGE migration patterns similar to those of “nonspecific” bands seen in HPAF lysates (Fig. 4A). These bands were not seen in irrelevant antibody immunoprecipitations of transfected NIH 3T3 cells (data not shown) or in the untransfected or H-ras-transfected NIH 3T3 cells (Fig. 6). The nature of these and their relationship to the RNP complex have not been determined; however, it is possible that they are processed forms or other proteins weakly associated with the complex. These additional bands were not seen in supernatants above the NIH 3T3 transfectants (data not shown). Subsequent experiments (see below) demonstrated that the antibodies bind to the M, 240,000 protein. The results of a larger assay analyzing supernatants, cytosol, and nuclei for these antigens are summarized in Table 1 and show that the M, 240,000 protein and the RNA moieties were found in the nuclei and in cytosolic lysates of all HPAF transfectants tested, including five different primary HPAF transfectants, three different secondary transfectants, and three tertiary transfectants. The supernatant form of the antigen was found in all of the same cell lines except one primary transfectant. The antigen was not found in untransformed NIH 3T3 cells from two sources, a spontaneously transformed NIH 3T3 cell line which we have isolated (1), or NIH 3T3 cells transformed with DNA from the EJ bladder carcinoma.

Several different human cell lines were originally tested for RNP antigen expression by immunofluorescence (1). It was subsequently found that some cell lines which were negative for antigen expression by immunofluorescence actually expressed small amounts of antigen which could be detected by immunoprecipitation. The results of screening a number of human cell lines by immunoprecipitation are also presented in Table 1.

Antibody Binding to the RNP Complex. None of the antibodies to the RNP antigen were able to immunoprecipitate the characteristic RNA bands from HPAF total cellular RNA that had been purified by either detergent and phenol/chloroform
Fig. 4. (7.5%) SDS-PAGE. A, HPAF cell lysates metabolically labeled with [35S]cysteine and [35S]methionine were immunoprecipitated by the following antibodies: lane 1, autoimmune human serum (anti-Ro, -La, -Sm); lane 2, autoimmune human serum (anti-Ro, -La, -Sm, -ribosomal RNP); lane 3, autoimmune human serum (anti-Sm, -ribosomal RNP); lane 4, autoimmune human serum (anti-Sm); lane 5, normal human serum; lane 6, irrelevant control murine monoclonal antibody (IgG1); lane 7, irrelevant control murine monoclonal antibody (IgG2b); lane 8, murine monoclonal antibody S1DII (IgG1); lane 9, murine monoclonal antibody 9F9 (IgG2b). These immunoprecipitations were performed as described in “Materials and Methods,” using Pansorbin and the indicated wash conditions. *, specific bands. B, immunoprecipitations of HPAF supernatant labeled with [35S]cysteine and [35S]methionine. Antibodies and methods as described in A. *, specific bands. Lane 9 was omitted in this experiment. C, immunoprecipitations of HPAF supernatant labeled with [33P]. Antibodies and methods as described in A.

Fig. 5. Monensin inhibits the appearance of the RNA in HPAF supernatants. (10%) SDS-PAGE of antibody S1DII immunoprecipitates of [35S]cysteine/methionine-labeled (lanes A-D) or 1.5% SDS-agarose gel electrophoresis of [33P]-labeled (lanes E-H) HPAF lysates and supernatants. Lanes A and E, 6-h labeled cell lysates without monensin treatment; lanes B and F, 6-h labeled cell lysates with 10 μM monensin; lanes C and G, supernatants without monensin; lanes D and H, supernatants with 10 μM monensin treatment. These immunoprecipitations were performed using goat anti-mouse second antibody and high salt radioimmunoprecipitation wash conditions, as described in “Materials and Methods.” These conditions do not permit visualization of the lower M, proteins.

Characteristics of the RNA Component. RNA extracted from immunoprecipitates (by phenol/chloroform extraction) was analyzed for homology to rRNA using a probe derived from Drosophila rRNA (8), which contains sufficient homology to human RNA to hybridize to the 28S rRNA band of HPAF total cellular RNA. As can be seen in Fig. 9, RNA purified from supernatant immunoprecipitates showed a mobility distinct from 18S and 28S rRNA in formaldehyde-agarose electrophoresis and did not bind the Drosophila rRNA probe in Northern blots under stringency conditions which permitted the binding of the rRNA probe to HPAF 28S rRNA.

DISCUSSION

This report describes a unique RNP complex defined by monoclonal antibodies raised against NIH 3T3 cells that were transformed by transfection with DNA from a human pancreatic adenocarcinoma cell line. Although the full stoichiometry of the association of this complex is not known, the data presented here suggest that these MAbs recognized a protein of M, 240,000 that is in association with at least two (and
Perhaps the most surprising finding regarding this antigen was that it showed both nuclear and cytoplasmic localization (Fig. 1) and was secreted from a number of cell lines (Fig. 5 and Table 1). Although the available evidence indicates that the antibodies recognize the protein moiety of the RNP complex (Fig. 8), the subcellular localization studies could detect both protein alone and protein bound to RNA. Our initial reaction to the apparent secretion of this complex was that we had raised antibodies to either a Mycoplasma or viral contaminant of our cultures. However, several independent lines of negative evidence (see “Results”) suggested that this was not the case.

Two hypotheses can be proposed as explanations for the secretion of the molecule from cells: either the secreted form has a biological function or it is an inactive metabolic byproduct. In either case, the mechanism whereby such a large molecular complex is assembled and transported across both nuclear and outer cell membranes and the potential functions of the complex either inside or outside the cell present fascinating questions for further study. One possibility for transport (supported by the monensin experiments) is that the complex is secreted by exocytosis into microvesicles, as has been hypothesized for a novel RNA-proteolipid reported by Wyszorek et al. (14, 15). These investigators have described an RNA proteolipid which was found in supernatant above a number of transformed human cell lines and in the serum of a variety of cancer patients but was not found in supernatants above normal cell lines or in a variety of serum samples from normal individuals and patients with benign diseases. The available molecular information on this complex indicated that the RNA showed 27S migration in density gradients and the associated protein had a molecular weight of 1250, determined by gel filtration. We have not estimated our RNPs size on density gradients; however, the larger RNA component reported here migrated faster than 28S ribosomal RNA and slower than 18S ribosomal RNA on agarose gels. Other similarities between these were their secretion into culture supernatants (both inhibitable by monensin) of transformed but not normal cell lines and the relative stability of the nondenatured RNA. The major dissimilarity between the reported characteristics was the size of protein molecule (М, 240,000 versus 1,250), which may or may not be explained by differences in the purification procedures (immunoaffinity methods versus gel filtration and density gradient centrifugation, either of which may have eliminated various components of the complex).

Fig. 6. (10%) SDS-PAGE of antibody S1D11 immunoprecipitates of [35S]-cysteine- and [38S]methionine-labeled from the following cell lines: lane A, NIH 3T3 primary transformant transfected with HPAF DNA; lane B, NIH 3T3 primary transformant transfected with HPAF DNA; lane C, NIH 3T3 secondary transformant transfected with primary transformant DNA; lane D, NIH 3T3 secondary transformant transfected with primary transformant DNA; lane E, NIH 3T3 tertiary transformant transfected with secondary transformant DNA; lane F, NIH 3T3 tertiary transformant transfected with secondary transformant DNA; lane G, untransfected NIH 3T3; lane H, NIH 3T3 cell line T-24, transformed with bladder carcinoma DNA (c-H-ros). These immunoprecipitations were performed as described in “Materials and Methods,” using goat anti-mouse second antibody and low salt radioimmunoprecipitation wash conditions. Isotype control antibody immunoprecipitates showed no bands. * bands of the same M, seen in Fig. 4, A and B.

possibly more) other proteins (М, 110,000 and 45,000) and two RNA molecules (1.5 and 3.0 kilobases). The size of the RNP components together with its secretion and cytoplasmic and nuclear subcellular distribution distinguishes this RNP from other RNPs heretofore described, including ribosomal elements (11), Sn RNPs (12), Hn RNPs (13) and m RNPs (13). In addition, the RNA moiety was shown to be distinct from rRNA by Northern blotting analysis (Fig. 9). This RNP shows some similarity to two other RNPs previously described (14–17), which will be subsequently discussed.

Fig. 7. (0.1%) SDS-5.5% agarose gel electrophoresis of antibody S1D11 immunoprecipitates (lanes 1) or an isotype control antibody immunoprecipitate (lanes 2) of [35S]orthophosphate-labeled lysates from the following cell lines: lanes A, NIH 3T3 primary transformant transfected with HPAF DNA; lanes B, NIH 3T3 primary transformant transfected with primary transformant DNA; lanes C, NIH 3T3 secondary transformant transfected with secondary transformant DNA; lanes D, NIH 3T3 tertiary transformant transfected with secondary transformant DNA; lanes E, NIH 3T3 tertiary transformant transfected with secondary transformant DNA; lanes F, NIH 3T3 tertiary transformant transfected with secondary transformant DNA; lanes G, NIH 3T3 cell line T-24, transformed with bladder carcinoma DNA (c-H-ros). These immunoprecipitations were performed as described in “Materials and Methods,” using goat anti-mouse second antibody and low salt radioimmunoprecipitation wash conditions. Eighteen S and 28S RNA migration was established by ethidium bromide staining of a separate lane containing HPAF total RNA.
Table 1

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<th>Cell line description</th>
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<td>Protein</td>
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* Number of cell lines positive for the M, 240,000 band by immunoprecipitation/total number of cell lines tested.

† Number of cell lines positive for the 1.5- and 3.0-kilobase RNA bands by immunoprecipitation/total number of cell lines tested.

‡ Cell lines tested: HPAF, T3M4, Capan 1, Colo 357, Mia PaCa, and QGP1.

§ Cell lines tested: K562, HL60, U937, Molt 4, HSB, CEM, Daudi, and REH.

‖ Cell lines tested: two primary cultures obtained locally (RSM and HUFF) and GM3498B.

PHA, phytohemagglutinin.

The RNP described herein also bears resemblance to a unique RNP purified by Kedersha and Rome (16) from rat liver microsomal coated vesicle preparations. The microsomal RNP contained a small RNA species (less than 5.8S) and several proteins with molecular weights of 210,000, 192,000, 104,000, 54,000, and 37,000. This RNP was expressed in a variety of cell types including murine 3T3 cells. Recently, this group (17) reported that the human form of the predominant protein found in this structure was M, 100,000. Several of the protein molecular sizes reported for this RNP were similar to the molecules seen in the RNP reported in this paper; however, the RNA components apparently had distinct sizes. If these complexes are the same, these distinctions may be the result of different purification and analysis procedures, as well as potential differences between the rat and human forms of the molecule. Homologies between these structures should be resolved by further characterization of both RNPs.

Two questions which will be addressed in further studies of the RNA and protein moieties include the nature of their physicochemical association and the reason for their coordinate expression in the transfected NIH 3T3 cells. The latter question is particularly intriguing because of the fact that the antibodies apparently bind to the M, 240,000 protein moiety of the antigen and yet immunoprecipitate both RNA and protein in transfected NIH 3T3 cells. This fact (that the RNA is also expressed in some transfected NIH 3T3 cells) raises several possibilities: (a) the human genes for the RNA and protein were both successfully transfected into the NIH 3T3 cells and are both expressed in the transformed transfecants; (b) the gene for the human protein (M, 240,000) was successfully transfected into the NIH 3T3 cells, which now binds to a murine equivalent of the human RNA moiety; or (c) the transformation of the murine NIH 3T3 cells with human HPAF DNA has induced the expression of a murine equivalent of the human RNA-protein.
The positions of the 18S and 28S HPAF ribosomal bands were determined by methylene blue staining of separate filters with lanes containing 5 µg of total cellular RNA. All samples were run on the same 0.8% agarose gel and transferred to nitrocellulose. Lanes A and B were probed with nick-translated pDmr 151*L, a ribosomal DNA probe derived from Drosophila melanogaster (8). The positions of the 18S and 28S HPAF ribosomal bands were determined by methylene blue staining of separate filters with lanes containing 5 µg of total cellular RNA. All samples were run on the same 0.8% agarose gel and transferred to nitrocellulose. Lanes A and B were hybridized with the probe at 35°C for 18 h in 40% formamide, 6x standard saline citrate, IX saline citrate for 2 h (four buffer changes). Lane C was autoradiographed directly following transfer.

In summary, this work has described a novel ribonucleoprotein particle that is secreted from a number of human cell lines and NIH 3T3 cell lines transfected with human pancreatic tumor DNA. Further elucidation of the molecular structure of the complex should provide insight into its functional role in the biology of normal cells and tumor cells.

REFERENCES


A Novel Ribonucleoprotein Complex Defined by Monoclonal Antibodies to NIH 3T3 Cells Transfected with Human Pancreatic Adenocarcinoma DNA

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