

High expression levels of *ras* p21 protein in normal mouse heart tissues

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We have investigated the levels of protein encoded by the *ras* oncogene in normal mouse tissues using an immunoblotting technique. We have found that heart from young or adult NIH or Balb C strains of mice contain high levels of *ras* protein as compared to lung, liver, spleen, kidney, brain and skeletal muscle tissues from the same animal. Our results indicate that cellular *ras* expression does not in every case correlate with cell proliferation.

A feature of cellular oncogenes is the extent to which these genes have been conserved across phylogenetic time. This strongly suggests that these genes serve important physiological functions. Studies with RNA isolated from embryos, from fetuses at different stages throughout mouse prenatal development and from a variety of extraembryonal and postnatal tissues have shown that sequences homologous to the Harvey *ras* oncogene are transcriptionally active at relatively high levels in all normal tissues examined (1-2). However, similar RNA hybridization studies have shown that expression of *ras* oncogenes is elevated in some premalignant and malignant tissues (3-6). Expression of *ras* proteins in a variety of mammalian cells and tissues has also been described (7-8). The *ras* p21 proteins encoded by members of the *ras* gene family have a molecular weight of 21 000 daltons, are localized in the plasma membrane and bind guanine nucleotides (9-11). Normal *ras* proteins have a GTPase activity which is reduced in the oncogenic variants (12-14) and activate yeast adenylate cyclase in the presence of guanine nucleotides (15). In the present study we have used an immunoblotting technique to examine *ras* protein expression in a variety of normal mouse tissues. We have found high levels of expression of *ras* p21 protein in heart tissue.

Materials and Methods

Monoclonal antibodies and sample preparation

Monoclonal antibodies YA6-172 and Y13-259 and their preparation have been described previously (17). For sample preparation, tissue was minced finely with scissors and resuspended at a concentration of 0.5 g/ml in RIPA buffer (0.1 M Tris HCl pH 8.0, 1 mM EDTA, 1% Triton X-100, 0.5% Na

deoxycholate, 0.5% SDS and 1 mM PMSF). After 1 h at 4°C, the cell debris was spun down at top speed in a microcentrifuge for 5 min and the supernatant was used in the subsequent polyacrylamide gel electrophoretic analysis.

Polyacrylamide gel electrophoresis

One-dimensional SDS-polyacrylamide slab gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (16). The acrylamide concentration was 12.5% in the separating gel and 5% in the stacking gel. SDS sample buffer contained 2.5% SDS, 2.5% β -mercaptoethanol, 0.1 M Tris-HCl pH 6.8 and 10% glycerol.

Immunoblotting

Polypeptides were transferred from SDS-polyacrylamide slab gels to nitrocellulose for detection with monoclonal antibodies as previously described (18). Briefly, after PAGE the gel was soaked for 30 min in transfer buffer (0.196 M glycine, 0.25 M Tris HCl, 20% v/v methanol). Polypeptides were transferred to 0.45 μ m nitrocellulose paper using a Transblot (Bio-Rad) apparatus at 50 V/250 mA overnight. The nitrocellulose filter was then treated as follows: all procedures were carried out at room temperature unless otherwise specified. 1. Wash buffer (WB 250 mM NaCl, 25 mM Tris HCl/pH 8.0) containing 0.25% gelatin and 10% ethanolamine for 1 h. 2. Wash buffer containing 0.1% Tween 20 for 5 min. 3. 1:300 dilution of monoclonal antibody in wash buffer containing 0.1% gelatin for 1 h. 4. Wash 5 times for 5 min each with WB containing 0.1% Tween 20. 5. Treat with rabbit anti-rat IgG diluted 1:150 in WB containing 0.1% gelatin, for 1 h. 6. Wash 5 times for 5 min each with WB containing 0.1% Tween 20. 7. Treat with 125 I-protein A (5 μ Ci) in 3D buffer (0.5% SDS, 1% Triton X-100, 1% Na deoxycholate) for 45 min. 8. Wash 8 times for 5 min each with wash buffer containing 0.1% Tween 20. 9. Dry in air, place in plastic bag and expose in Kodak X-Omat RP film at -70°C .

Results

Immunoblotting of *ras* p21 protein in NIH mouse tissues revealed that polypeptides in heart tissue bind the Harvey and Kirsten specific (17) anti-p21 monoclonal antibody Y13-259 (Fig. 1). The pattern of p21 expression in lung, liver, heart, kidney and brain tissue was similar in 10 day or 3 month old NIH mice. Only heart tissue contained substantial levels of p21 protein. As a positive control we used a rat cell line (RFAGT1-1) obtained after transformation of rat 208F fibroblast cells with the recombinant plasmid pAGT1 (19) carrying the *Ha-ras1* oncogene isolated from the T24 bladder carcinoma cell line. RFAGT1-1 cells express high levels of *ras* p21 protein. The p21 protein encoded by the T24 *Ha-ras1* gene migrates slower than the normal *Ha-ras1* protein because replacement of gly (aa 12) with valine in the T24 protein gives an altered conformation (22). The bands with a molecular weight higher than p21 represent non-specific background as discussed below.

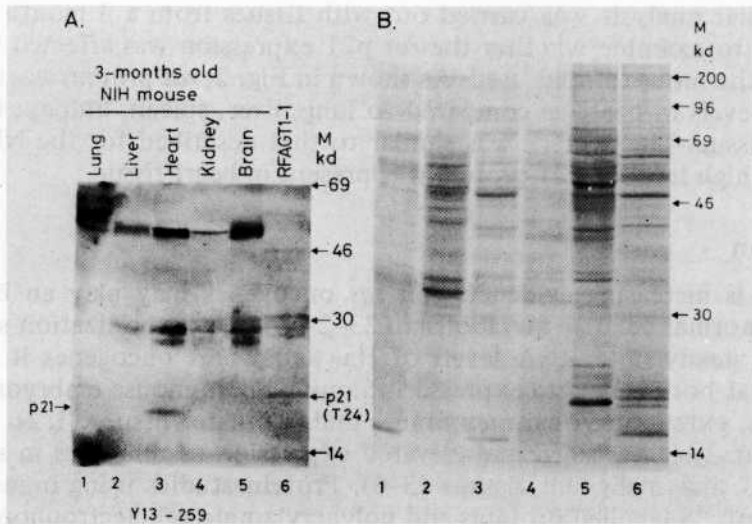


Fig. 1. Immunoblot analysis of *ras* p21 proteins in NIH mouse tissues with Y13-259 monoclonal antibody. (A) Autoradiogram, (B) gel stained with Coomassie blue.

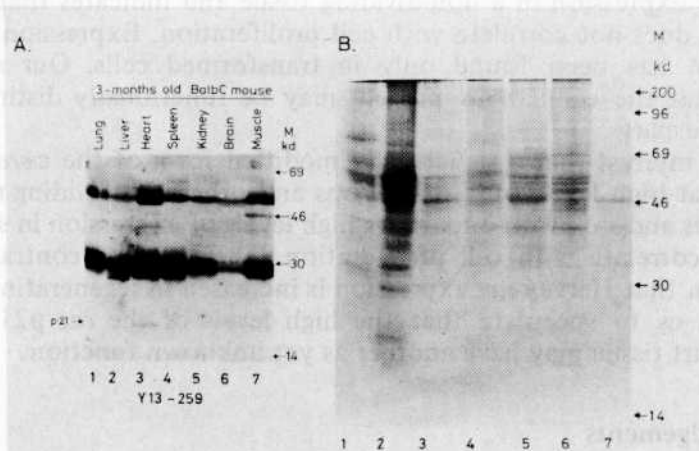


Fig. 2. Immunoblot analysis of *ras* p21 proteins in Balb C mouse tissues with Y13-259 monoclonal antibody. (A) Autoradiogram, (B) gel stained with Coomassie blue.

The same result was obtained with a Harvey *ras* p21 specific (17) monoclonal antibody YA6-172 (data not shown). Neither the p21 polypeptides nor any other proteins were labelled in a control experiment with preimmune rat serum in this assay. As an additional control we used GP 114.3 antiglycophorin monoclonal antibody (20), a rat IgG monoclonal antibody non-related to p21. Glycophorin which is normally expressed in erythroid cells (20) gave only the same non-specific bands observed with the *ras* p21 monoclonals (data not shown).

A similar analysis was carried out with tissues from a 3 month old Balb C mouse to examine whether the *ras* p21 expression was affected by differences in the mouse strains used. As shown in Fig. 2, *ras* protein was expressed at high levels in heart as compared to lung, liver, spleen, kidney, brain and muscle tissue. The pattern was similar to that described for the NIH mouse in that a high level of p21 protein was present in heart tissue.

Discussion

There is increasing evidence that *ras* oncogenes may play an important role in normal cellular metabolism (23-24). Using hybridization studies to measure steady-state RNA levels of Ha- and Ki-*ras* oncogenes it has been found that both genes are expressed ubiquitously in mouse embryos, fetuses, placentas, extraembryonal membranes and postnatal tissues (1, 2). A similar type of analysis has suggested elevated expression of *ras* genes in some pre-malignant and malignant tissues (3-6). Protein studies using immunoprecipitation of ³⁵S-labelled proteins and polyacrylamide gel electrophoresis (7, 8) or immunohistochemical methods (25) have given similar results.

In the present study we have used an immunoblotting method to examine *ras* protein expression in a variety of normal mouse tissues. Our results show elevated levels of *ras* proteins in heart tissue. This is the first demonstration of high *ras* expression in a non-dividing tissue and indicates that cellular *ras* expression does not correlate with cell proliferation. Expression of the viral *ras* protein has been found only in transformed cells. Our results here indicate that the cellular *ras* protein may be functionally distinct from its viral counterpart.

It is of interest that a structurally modified form of the *c-src* product is expressed at high levels only in neurons and other non-dividing tissues (26). Thus for *ras* and *src* proto-oncogenes high levels of expression in some tissues does not correlate with cell proliferation. These results contrast with the observation that Harvey-*ras* expression is increased in regenerating liver (27). This leads us to speculate that the high levels of the *ras* p21 protein in normal heart tissue may have another as yet unknown function.

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