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# Rearrangement and overexpression of the gene coding for tumor antigen p53 in a rat histiocytoma AK-5

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The gene coding for the cellular tumor antigen p53 is rearranged and overexpressed in a rat histiocytoma, AK-5. The protein coded by the gene was detected by immunofluorescence and its full size was confirmed by immunoprecipitation using monoclonal antibodies against p53. Southern hybridizations with a full length cDNA probe specific for p53 indicated rearrangement of the gene. Alterations in the upstream region, which probably disrupt the normal regulatory control are suggested by the pattern obtained using a 5'-specific p53 probe in Southern hybridization.

Rearrangement; Overexpression; p53; Macrophage-like cell line

## 1. INTRODUCTION

The cellular tumor antigen p53 is a phosphoprotein which is detected at elevated levels in a wide variety of transformed cell lines [1,2]. This protein is present in normal cells and has a very short half-life. p53 was initially detected in SV-40-transformed cells, where it complexes with the large T antigen [3,4]. The half-life of the protein in complex form is much longer compared to p53 from normal cells. The gene coding for p53 has been mapped [5], cloned from various sources such as rat, mouse, human, etc., sequenced and compared [6–8]. The conservation of the sequence suggests an important function for the protein. Though the exact function of p53 is unclear, remarkably elevated levels of p53 in transformed and actively dividing normal cells indicate its possible involvement in normal cell proliferation, whereas elevated levels of mutated p53 have been correlated with cellular transformation [9].

The AK-5 is a rat histiocytic cell line possessing many of the macrophage-like characteristics [10]. This tumor arose in the peritoneal cavity of one of the rats when cell free ascitic fluid from ZAH (Zajdela ascitic hepatoma) [11] was injected into normal inbred animals. In an attempt to understand the factor(s) involved in the origin of the tumor and maintenance of the malignant phenotype, we observed the presence of tumor antigen p53 in AK-5. We report here our results regarding the rearrangement and overexpression of the gene coding for p53 in AK-5 cells.

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## 2. MATERIALS AND METHODS

### 2.1. *Animals and tumor*

AK-5 and ZAH tumors are maintained as ascites in an inbred colony of Wistar rats of 6–8 weeks. About $(4–6)\times 10^6$ tumor cells are injected i.p. and the animals die between day 6 and day 12.

### 2.2. *Immunofluorescence and immunoprecipitation*

Washed AK-5 cells were incubated with different monoclonals against p53 (pAb 122 and 421), which recognize epitopes situated at carboxy terminus [12], for 2 h at 4°C. The cells were washed and treated with 1:200 diluted FITC-conjugated antimouse Ig for 1 h. The cells were washed and observed for fluorescence.

Immunoprecipitations were done using pAb 122 and 421 monoclonal antibodies. Washed AK-5 cells ($10^7$) were resuspended in 3 ml of methionine free DMEM supplemented with 10% dialyzed fetal calf serum. Cells were preincubated for 1 h and subsequently incubated for 3 h at 37°C with 75 $\mu$Ci of [$^{35}$S]methionine (Amersham), washed with PBS and extracted into 200 $\mu$l of lysis buffer (10 mM phosphate buffer (pH 7.5), 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 20 mM PMSF) at 4°C for 45 min. Cell lysates were cleared by high speed centrifugation and adsorption onto protein A-Sepharose and non-immune serum. Equal amounts of radioactive proteins were immunoprecipitated with monoclonal antibodies against p53. Antibody-antigen complexes were separated by protein A-Sepharose treatment, resolved by SDS-PAGE and analyzed by autoradiography.

### 2.3. *Northern and Southern hybridizations*

Total RNA samples from normal liver, macrophages, AK-5 and ZAH were run on 1% agarose gel under denaturing conditions, transferred onto nitrocellulose paper and hybridized [13] with nick translated p53 cDNA probe [14]. After stringent washings (0.5 × SSC, 0.1% SDS) the blots were exposed for autoradiography. Similarly, DNAs from AK-5, ZAH, as well as normal liver were digested with *Bam*HI/*Eco*RI, size-fractionated by electrophoresis through 1% agarose, transferred onto nitrocellulose paper and baked for 2 h at 80°C as described by Southern [15]. Blots were hybridized with nick translated p53 cDNA probe at 60°C overnight (5 × Denhardt's solution, 5 × SSPE, 0.1% SDS and 100 $\mu$g/ml of sheared calf thymus

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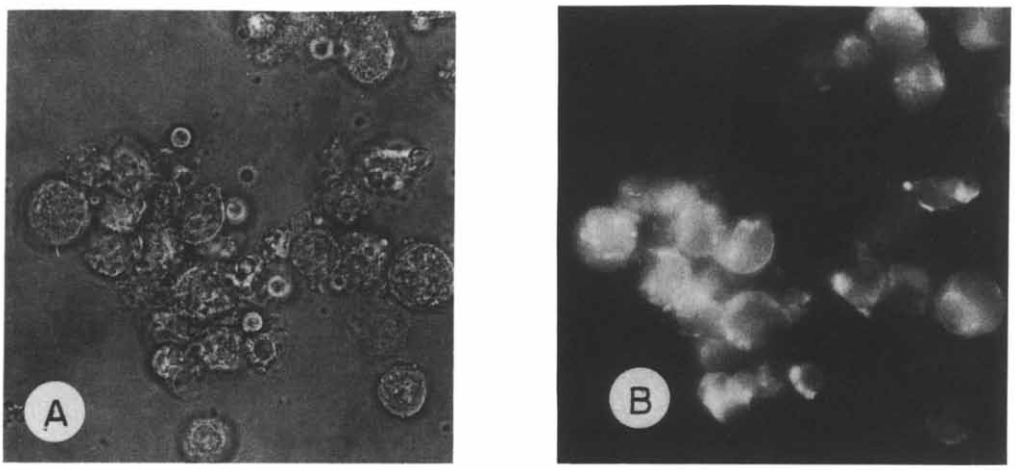


Fig. 1. Immunofluorescence of AK-5 cells with 421 monoclonal antibody. (A) Phase contrast; (B) FITC-stained cells; ×500.

DNA), washed three times with 2×SSC at 65°C for 5 min and two or three times with 0.2×SSC at 65°C for 15 min each, air dried and exposed to X-ray film at −70°C.

## 3. RESULTS AND DISCUSSION

The preliminary immunofluorescence experiments with two different monoclonal antibodies (pAb 122 and 421) against p53 indicated the presence of the protein in AK-5 cells (Fig. 1). In subsequent immunoprecipitations, these antibodies precipitated full length p53 protein from AK-5 cells (Fig. 2).

To investigate the level of expression of mRNA transcripts specific for p53 in AK-5, molecular hybridization experiments using a p53 cDNA clone were carried out. The results indicated the presence of p53 message in normal as well as transformed cells (Fig. 3A). However, the level of the message in AK-5 is higher compared to normal tissues and ZAH (20-fold higher compared to normal macrophages as estimated by densitometric scanning). The stability of the message was established by performing the Northern hybridizations using a cDNA probe for actin (Fig. 3B).

Chromosomal translocations or rearrangements within genomic DNA have been implicated in altered pattern of gene expression [16]. In order to understand the mechanism of overexpression of the p53 gene in AK-5, genomic DNA from AK-5 was analyzed using Southern hybridizations. The autoradiograms indicated presence of an additional fragment (1.2 kb) or an insertion within the gene, which is absent in the control DNA from normal liver and in the hepatoma (Fig. 4). These experiments were repeated with different restriction enzymes such as *Hin*dIII, *Pst*I, etc., and the pattern obtained with AK-5 DNA was consistently different compared to that of normal liver and ZAH DNA, whereas the hybridization pattern was comparable between normal liver and ZAH. These observations eliminated the possibility of polymorphism at the locus.

Southern hybridizations were also performed using another cDNA probe which corresponded to the 5′ region of the p53 gene and the results obtained confirmed our earlier observations regarding the rearrangement of the p53 gene in AK-5 (Fig. 5).

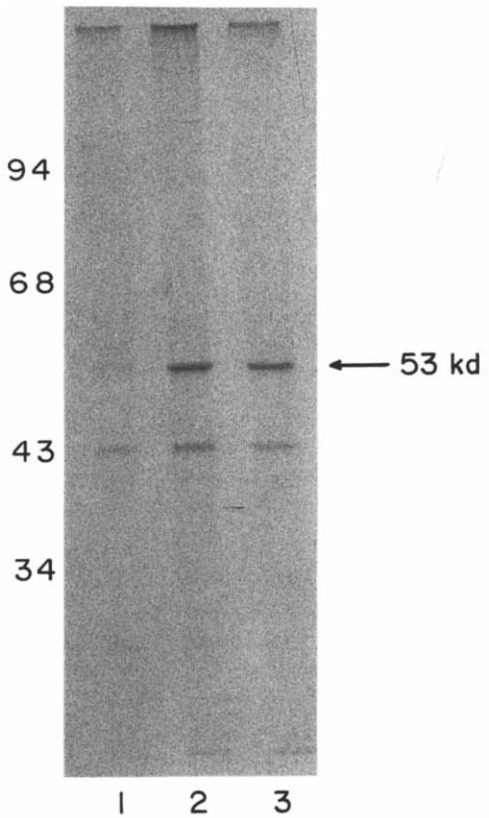


Fig. 2. Immunoprecipitation of [$^{35}$S]methionine-labelled proteins with anti p53 antibody. Labelled proteins were immunoprecipitated with anti p53 antibody as described in the text. The precipitates were analyzed by SDS-PAGE. Lanes: (1) normal rat serum; (2) pAb 421; (3) pAb 122.

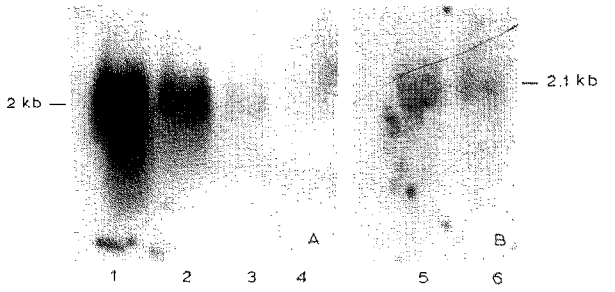


Fig. 3. Northern hybridization analysis of total RNAs from AK-5, ZAH, rat liver and macrophages. (A) Hybridization with p53 cDNA. Lanes: (1) AK-5; (2) ZAH; (3) liver; (4) macrophage. (B) Hybridization with actin cDNA. Lanes: (5) AK-5; (6) liver.

Recent studies have demonstrated that wild-type p53 is not a transforming protein, while mutant species of p53 which contain a single amino acid change within a region between amino acids 130 and 234, can cooperate with ras to transform primary rat embryo fibroblasts in a cotransfection experiment [17,18]. Rearrangement of p53 gene is shown to be involved in development of Friend virus induced erythroleukemia, resulting either in the inactivation of p53 [19] or the synthesis of elevated levels of antigenically related truncated polypeptides [20]. Rearrangement of p53 gene has been demonstrated in certain human osteogenic sarcomas [21]. Due to its ability to suppress transformation upon overexpression, protooncogene p53 is classified as recessive oncogene, which on inactivation either by mutation or overexpression due to rearrangement seems to render the cells susceptible to uncontrolled growth.

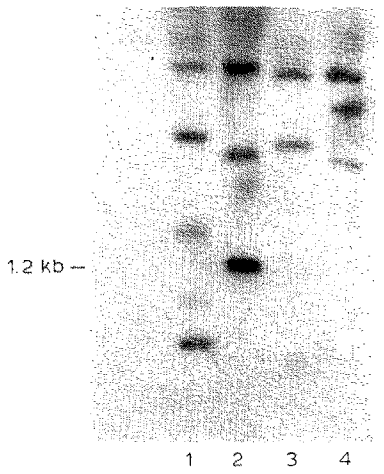


Fig. 4. Southern hybridization with full length cDNA probe specific for p53. Genomic DNAs from tumor cells and normal tissues were digested, size fractionated and transferred onto nitrocellulose paper. Hybridizations were carried out with nick translated cDNA probe specific for p53 under stringent conditions. Lanes: (1) AK-5 DNA digested with *Eco*RI; (2) AK-5 DNA digested with *Bam*HI; (3) liver DNA digested with *Eco*RI; (4) liver DNA digested with *Bam*HI.

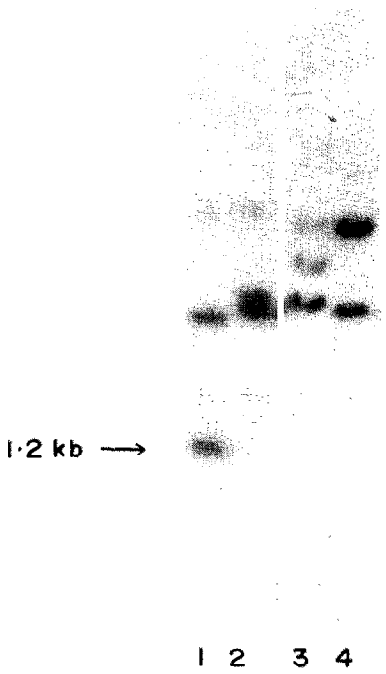


Fig. 5. Southern hybridization with a cDNA probe specific for the 5′ region of p53. Lanes: (1) AK-5 DNA digested with *Bam*HI; (2) liver DNA digested with *Bam*HI; (3) AK-5 DNA digested with *Hin*dIII; (4) liver DNA digested with *Hin*dIII.

Our observations regarding expression of p53 in AK-5 can be summarized as follows. First, cellular tumor antigen p53 is produced by a histiocytoma in detectable amounts. Second, the protein is full length and stable as it is recognized by monoclonal antibodies against p53 in immunoprecipitations and Western blot experiments. Third, the message specific for p53 is overexpressed in AK-5 without any alteration in the transcript size. Fourth, using a full length cDNA probe and a probe specific for the 5′ end of the p53 gene, rearrangement of the gene can be detected in Southern hybridizations.

Our observations suggest that the rearrangement has altered the 5′ region of the p53 gene which is crucial with respect to its pattern of expression. The probe specific to the 5′ region of the gene spans the first 126 bases of the structural gene. Detailed characterization and comparison of the 5′ region sequence from human, rat and murine p53 gene have revealed a crucial role for the 5′ region of the gene with respect to modulation of the expression [22]. Disruption of the negative transcription control situated upstream of the promoter may result in the overproduction of the message specific for p53. In AK-5, the overexpression of the p53 protein which is the product of a specifically rearranged gene, may be providing a selective growth advantage to the cells for uncontrolled growth.

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