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Regulation of ribosomal protein S25 in HL60 cells isolated for resistance to adriamycin

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The ribosomal protein S25 gene is highly overexpressed in HL60 cells isolated for resistance to adriamycin. In contrast there is no overexpression of 3 other ribosomal genes which code for proteins S14, S17 and S24. Studies with an antibody against a synthetic peptide of the S25 protein show that although the S25 gene is overexpressed in resistant cells there is no corresponding increase in the levels of S25 protein. These results suggest that the r-protein levels are highly regulated by translational controls or protein turnover.

Ribosomal protein S25; Gene overexpression; Adriamycin; Drug-resistance

1. INTRODUCTION

HL60 cells isolated from resistance to adriamycin are multidrug resistant and defective in the cellular accumulation of drug [1]. These cells do not however contain detectable levels of P-glycoprotein [2], a protein which contributes to drug resistance in a number of other isolates [3,4]. Recent studies have demonstrated that HL60/Adr cells do however contain increased levels of a 190 kd protein (P-190) which exhibits a minor sequence homology with P-glycoprotein [5]. The exact role of P190 in drug resistance in HL60 cells remains to be determined. Previously we have carried out studies to identify genes which may be overexpressed in resistant cells. The identity of these genes could be of considerable importance in clarifying the molecular basis of HL60/Adr drug resistance. In these studies we used a subtractive hybridization approach to prepare a cDNA library from resistant cells [6]. A clone generated by this library was isolated and found to represent a cDNA of the human ribosomal S25 gene [7]. In the present study we have used this cDNA as a probe to analyze S25 gene expression in drug sensitive and resistant cells.

2. MATERIALS AND METHODS

2.1. *Cell culture*

HL60 cells isolated for resistance to adriamycin were prepared as described previously [1]. Both sensitive and resistant cells were grown in RPMI medium supplemented with 10% fetal bovine serum.

Abbreviations: cDNA, DNA complementary to mRNA; r-protein, ribosomal subunit protein; HL60/ADR, HL60 cells isolated for resistance to adriamycin

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2.2. *Isolation of ribosomal proteins*

Human r-proteins were isolated using a modified procedure described previously [8]. Cells were suspended in buffer containing 30 mM Tris-HCl, (pH 7.5), 125 mM KCl, 5 mM $Mg(OAC)_2$, and 10 mM 2-mercaptoethanol. Cells were lysed by the addition of Nonidet P40 to a final concentration of 0.05%. After removal of nuclei, the supernate was placed on top of a 3 ml solution of 1 M sucrose. The polysomes were recovered after centrifugation in a SW50.1 rotor for 6 h at 35,000 rpm. The pellet was treated with glacial acetic acid, and the proteins were precipitated with acetone. The r-proteins were reduced with dithio-erythritol and alkylated with iodo-acetamide.

2.3. *Isolation of mRNA associated with polysomes*

Polysomes were isolated as described above. The polysome pellet was suspended in buffer containing 30 mM Tris-HCl (pH 7.4), 1% SDS, 100 mM NaCl, 10 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM EDTA, and 7 M urea. The mRNA was extracted with phenol/chloroform and precipitated with ethanol.

2.4. *Northern blot analysis*

Total cellular RNA was isolated from sensitive and resistant cell [8] and subjected to electrophoresis in a 1.2% agarose gel containing 20 mM morpholinopropanesulfonic acid (pH 7.0) and 2.2 M formaldehyde. The RNA was transferred to Gene Screen Plus and hybridization with radioactive S25 cDNA [9] was conducted at 65°C for 14 h in a solution containing 1 M NaCl, 0.1% SDS, 0.1 M sodium phosphate, 0.3 mg/ml of herring sperm DNA. After hybridization, the membrane was washed at 65°C twice for 20 min in 2 × SSC/0.1% SDS and once for 20 min in 0.5 × SSC/0.1% SDS.

2.5. *Peptide synthesis and immunization*

Peptides were synthesized according to the deduced sequence of human r-protein S14 [10] and S25 [7] by the Merrified solid-state method [11]. The sequences of the peptides which were used to make antisera are PSDSTRRKGGRRGRR for S14 and RNTKGGDA-PAAGEDA for S25. The synthetic peptides containing lysine at the amino terminus were conjugated to keyhole limpet hemocyanin, emulsified with Freund's complete adjuvant, and injected subcutaneously into New Zealand white rabbits. Antisera were collected after four injections of conjugated peptide.

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2.6. *Western blot analysis*

Proteins were separated by SDS-PAGE on a 12% polyacrylamide gel and transferred to nitrocellulose paper as described by Towbin [12]. The blots were incubated in a blocking solution containing 0.02 M phosphate buffer (pH 7.4), 0.15 M NaCl, 1% bovine serum albumin, 0.05% Tween 20 (PNBT) for 2 h at 37°C. The immunoblot reaction was carried out with antisera diluted in PNBT overnight at room temperature. After washing with PNBT the blots were incubated with [^{125}I]protein A (5×10^5 cpm/ml) for 90 min at room temperature and washed again. The blots were exposed to X-film at −20°C.

3. RESULTS AND DISCUSSION

A cDNA clone coding for human r-protein S25 has been isolated from a subtractive library of HL60/Adr cells and subcloned in plasmid PGEM-11Zf [7]. The S25 cDNA has been used as a probe to examine gene expression in adriamycin-resistant and sensitive cells. The Northern blots demonstrate that the S25 gene was highly overexpressed in the resistant cells as compared to sensitive cells (Fig. 1, lanes 7 and 8). In contrast to S25, the levels of mRNA expression of r-protein genes S14 [10], S17 [13] and S24 [14] are essentially identical in sensitive and resistant cells (Fig. 1, lanes 1–6). This finding suggests that S25 represents a specific r-protein gene which is overexpressed in HL60 cells isolated for resistance to adriamycin.

mRNA isolated from the polysomes of sensitive and resistant cells was examined by Northern blot analysis. The mRNA levels associated with ribosomes were compared with total cytoplasmic mRNA. The results demonstrate that the levels of total cytoplasmic mRNA (Fig. 2, lane 2) and polysome associated S25 mRNA (Fig. 2, lane 4) or resistant cells are considerably increased compared to the corresponding mRNA of sensitive cells (Fig. 2, lanes 1 and 3). A densitometric trace of the autoradiogram shows that cytoplasmic and polysome associated resistant mRNA is increased 3.9- and 2.8-fold, respectively over that in sensitive cells. In a parallel experiment there is no detectable increase in either total cytoplasmic or ribosome associated S14 mRNA in resistant cells (data not shown).

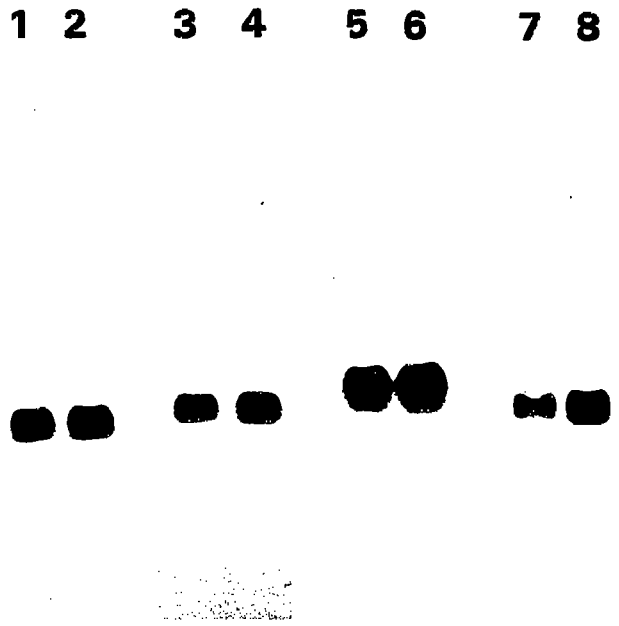

Fig. 1. RNA blot analysis of r-protein gene expression in sensitive and resistant cells. Total RNA isolated from HL60/sensitive (lanes 1, 3, 5 and 7) and HL60/resistant (lanes 2, 4, 6 and 8) was examined by Northern blot analysis as described in section 2. Gene expression was analyzed using cDNA probes which correspond to r-proteins S14 (lanes 1 and 2), S17 (lanes 3 and 4), S24 (lanes 5 and 6), and S25 (lanes 7 and 8).

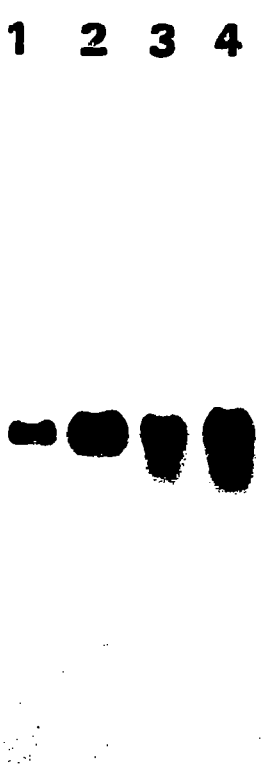

Fig. 2. Analysis of mRNA associated with ribosomes in sensitive and resistant cells. Total RNA was isolated from HL60/sensitive (lane 1) and HL60/resistant cells (lane 2). RNA was also isolated from HL60/sensitive ribosomes (lane 3) and HL60/resistant ribosomes (lane 4). The mRNA was analyzed using the radioactively labeled S25 cDNA probes.

The levels of r-proteins S14 and S25 in sensitive and resistant cells were examined by Western blot analysis using antisera against synthetic peptides which correspond to the deduced sequences of these proteins (Fig. 3). These proteins were examined in both cytoplasm (Fig. 3A) and in isolated ribosomes (Fig. 3B). The results demonstrate that the levels of S14 and S25 proteins in cytoplasm and ribosomes of sensitive and resistant cells are essentially identical (Fig. 3).

Previous studies have shown that the balanced synthesis of each r-protein is carefully regulated by the cell to provide an equal ratio necessary for the assembly of the ribosomes [15]. Our results show that although there is a 2.8-fold increase of S25 mRNA associated with

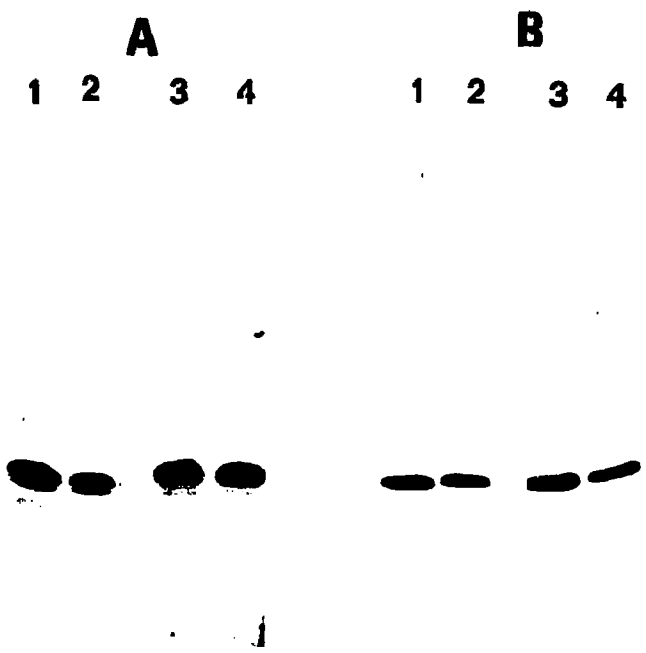

Fig. 3. Immunoblot analysis of S25 and S14 proteins. Cytoplasmic proteins and ribosomal proteins were separated by SDS-PAGE. Western blot analysis using antisera to S14 and S25 synthetic peptides was carried out as described in Methods. Panel A: cytoplasmic proteins were prepared from HL60/sensitive (lanes 1 and 3) and HL60/resistant (lanes 2 and 4). Panel B: ribosomal proteins were prepared from HL60/sensitive (lanes 1 and 3) and HL60/resistant (lanes 2 and 4). The filers containing transferred proteins were incubated with antiserum against S14 peptide (lanes 1 and 2) and S25 peptide (lanes 3 and 4).

ribosomes in adriamycin-resistant cells there is no parallel increase in the level of the S25 protein. Several experiments have been performed to examine the mechanisms involved in regulating levels of r-proteins [16,17]. The levels of these proteins appear to be regulated by both the efficiency of translation [16] and the rate of turnover [18]. Previous studies have shown that cells containing increased levels of r-protein S10 mRNA results in overproduction of the S10 protein. Excess levels of this protein occurred only transiently since S10 protein was found to turn over very rapidly, with a half-life of approximately 5 min [18]. The results obtained in this study suggest that the levels of S25 protein in the resistant cells may also be regulated by both the efficiency of translation and protein turnover.

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