SHORT COMMUNICATION

Evidence that multidrug resistance in Chinese hamster ovary cells is associated with alterations in the endoplasmic reticulum

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Multidrug resistance (MDR) in animal and human cell lines is often associated with a reduction in cellular accumulation of the drugs concerned (Kessel et al., 1968; Dano, 1973). Drug transport studies have shown that this is a facet of altered translocation of drugs across the plasma membrane which may involve both decreased drug penetration and/or increased drug eflux (Skovsgaard, 1978; Inaba et al., 1981). Many studies have therefore focussed on the composition of this organelle in order to identify the molecular basis of drug resistance (for review see Riordan & Ling, 1985). By far the most striking membrane alteration in several MDR cells is the presence of a high molecular weight glycoprotein family (P-glycoprotein) (Juliano & Ling, 1976; Biedler & Peterson, 1981; Kartner et al., 1983). In addition to P-glycoprotein hyperexpression, a small acidic cytosolic protein called V19 (Meyers & Biedler, 1981), CP22 (Koch et al., 1986) or Sorcin (Van der Bleek et al., 1986), has also been noted in some hamster and mouse MDR cell lines. Although the role of this protein is unknown, it is able to bind calcium with high affinity (Koch et al., 1986) suggesting that altered calcium metabolism may also be a component of the MDR phenotype. The present study indicates that in addition to hyperexpression of V19/CP22/Sorcin and P-glycoprotein, structural changes in the endoplasmic reticulum (ER) also occur in MDR CHC³ cells.

The adenosine-, thymidine- and glycine-requiring auxotroph AUXBI of CHO cells and its colchicine-resistant mutant CHC³ were obtained from Dr Ling of the Ontario Cancer Institute, Toronto, Canada. Cells were grown in suspension in minimal Essential Medium containing 10% foetal calf serum, streptomycin and penicillin. Membrane isolation was either by use of a Stansted cell disruptor, exactly as described by Riordan and Ling (1979), or by ultrasonic disintegration for 5 sec at 4°C using a MSE sonicator, model 1276, amplitude setting 22 microns. After disruption or sonication the following differential centrifugation steps were applied: nuclear spin, 300 g for 10 min at 4°C; mitochondrial spin, 4000 g for 10 min at 4°C; microsomal spin, 35,000 g for 30 min at 4°C. The resulting microsomal pellet was either dissolved in 0.1% SDS prior to protein determination and gel electrophoresis or was applied to a discontinuous sucrose gradient consisting of 60% (w/v), 45%, 31% and 16% sucrose, and centrifuged at 76000 g for 1 hr according to Riordan and Ling (1979). Material banding at the three interfaces was collected, washed and solubilised in 0.1% SDS. Aliquots of whole cell homogenate were also solubilised in 0.1% SDS. Protein determinations were according to Lowry et al. (1951).

SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli (1970) and employed a 10% SDS gel run under reducing conditions. Gels were stained with PAGE blue 83 (BDH Chemicals Ltd, Poole, UK). Transfer of resolved proteins from SDS gels to nitrocellulose filter paper was essentially as described by Towbin (1979). Protein transfer was performed for 4 hr at 4°C at a constant current of 0.5 A using a solution containing 0.0125 M Tris-base, 0.2 M glycine (pH 8.5) and 20% methanol as electrode buffer. Prior to immunoblotting additional protein binding sites on the nitrocellulose filter paper were blocked by incubation in 5 mM EDTA, 0.25% gelatin, 0.01 M NaCl, 0.15 M NaCl, 0.05 M Tris base and 0.05% NP40. The nitrocellulose paper was then incubated overnight with monospecific affinity purified antibody to endoplasmic (Koch et al., 1987). After washing ¹²⁵I protein A autoradiography was used to visualise antibody binding to protein bands.

The method for the detection of calcium binding proteins by ⁴⁴Ca autoradiography on nitrocellulose paper was according to Maruyama et al. (1984). After transfer of cytosolic proteins, the paper was soaked in a solution containing 60 mM KCl, 5 mM MgCl₂ and 10 mM Tris-HCl (pH 6.8) prior to incubation in the same buffer containing 0.5 mCi ⁴⁴CaCl₂ (Amersham International, Aylesbury, UK) for 10 min. The nitrocellulose paper was rinsed in distilled water for 5 min, dried and autoradiographed.

Analysis of microsomal membrane proteins from CHC³ and AUXBI cells by SDS gel electrophoresis revealed increased levels of a protein having a relative molecular mass of 92 kDa in the drug resistant line (Figure 1). This increase was observed in microsomal membranes prepared either by sonication or by use of a Stansted cell disruptor. Conversely, levels of the 92 kDa protein were consistently greater in the

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soluble fraction (cytosol) from AUXBI cells than in that from CH³C⁴ cells. Gel electrophoresis of microsomal membranes banding at the 31–45 and 45–60 sucrose density interfaces similarly revealed increased levels of the 92 kDa protein in the drug resistant cell line suggesting that the observed change in the level of the 92 kDa protein in the microsomal membranes of resistant CHO cells following disruption does not result from differences in the purity of microsomal material produced by sonication or by mechanical disruption. Gel electrophoresis failed to show the presence of this protein in plasma membrane-derived material banding at the 16–31 sucrose density interface.

Analysis of whole cell homogenates by gel electrophoresis showed no difference in the overall expression of the protein in CH³C⁴ cells and AUXBI cells.

Figure 2 shows that the major protein band recognised by the monospecific affinity-purified anti-endoplasmin antibody was the 92 kDa protein. Densitometry was used to confirm that following disruption, significantly more of this protein is associated with the microsomal membranes from CH³C⁴ cells than with those from AUXBI cells, that there is much less of this protein in the microsomal supernatant from CH³C⁴ cells than from AUXBI cells, and that the total amounts of this protein in whole cell homogenates is no different in the two cell types.

When microsomal membranes from CH³C⁴ cells were probed for Ca²⁺ binding proteins after SDS gel electrophoresis and electroblotting, the 92 kDa protein was the only protein capable of binding Ca²⁺ in the micromolar range (Figure 3).

It is highly likely that the 92 kDa protein described in this study is the major glycoprotein, endoplasmin (Koch et al., 1987), based on its reactivity with an affinity purified antibody to endoplasmin and on its ability to bind Ca²⁺. Endoplasmin has been shown to be localised to the endoplasmic reticulum and is the same as one of the major stress related proteins GRP94 (Koch et al., 1987).

Immunoblotting analyses show that overall expression of

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Figure 2 Western blot analysis of endoplasmin content in whole cell homogenates and subcellular fractions from AUXBI and CH³C⁴ cells. 100 μg protein were analysed by SDS-gel electrophoresis and immunoblotting with antibody to endoplasmin (top half of blot). The 92 kDa band (indicated by the arrow) is the major immunopositive band. Tracks 1 and 2 AUXBI and CH³C⁴ respectively, whole cell homogenates prepared by Stansted cell disruption; Tracks 3 and 4 AUXBI and CH³C⁴ respectively, whole cell homogenates prepared by sonication; Tracks 5 and 6 AUXBI and CH³C⁴ respectively, whole cell homogenates prepared by Stansted cell disruption; Tracks 7 and 8 AUXBI and CH³C⁴ respectively, microsomal membranes prepared by Stansted cell disruption, Tracks 9 and 10 AUXBI and CH³C⁴ respectively, cytosol extracts prepared by Stansted cell disruption. The lower half of the blot has been reacted with affinity purified antibody to the cytosolic, drug resistance associated protein CP22.

Figure 3 Binding of Ca²⁺ to 92 kDa protein in microsomal membranes (track 1) and the cytosol fraction (track 2) from CH³C⁴ cells. The affinity of the 92 kDa protein for calcium is comparable to that of the calcium binding protein, CP22.
endoplasmic reticulum in multi-drug resistance. The authors would like to thank Jonathan Shaw, Norma Fox and Karen Wright for their technical assistance.

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