Volume 229, number 2, 329–332 FEB 05659 March 1988

The tissue dependent expression of hamster P-glycoprotein genes

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Received 7 January 1988

Using a sensitive RNase protection assay, we have measured the levels of P-glycoprotein mRNA in fourteen different Chinese hamster tissues and in three cell lines to determine whether a relationship exists between the level of P-glycoprotein expression and the occurrence of primary and acquired multidrug resistance (MDR) in cancer. P-Glycoprotein mRNA was detected in all tissues, suggesting that the protein is a normal constituent of the cell. High levels of P-glycoprotein mRNA were found in oesophagus, testis and uterus. Intermediate levels in brain, lung and ovary, and a very low level in the adrenal gland, bladder, bone marrow, heart, kidney, liver and spleen. There is no obvious correlation between this expression pattern and the occurrence of primary or acquired MDR.

P-Glycoprotein; Tissue specific expression; Multidrug resistance

1. INTRODUCTION

Chemotherapy is often not effective in the treatment of cancer, as some tumors are intrinsically resistant to chemotherapeutic agents (primary drug resistance), whereas other tumors can acquire resistance (secondary drug resistance). Treatment with one drug may result in the acquisition of resistance to a wide variety of drugs, including structurally and functionally, unrelated drugs (review [1]). This multidrug resistance (MDR) is mediated by the overexpression of a 170 kDa membrane protein, the P-glycoprotein [2–4]. This protein helps to keep the intracellular drug concentration low, probably by an active transport of drugs out of the cell [1]. Sequence analysis of human, mouse and hamster P-glycoprotein cDNAs has revealed a high degree of homology of the P-glycoprotein to bacterial transport proteins [3,5,6], supporting the hypothesis that the P-glycoprotein is involved in the active transport of drugs across the cell membrane (review [7]). Since not all tumors are equally sensitive to drugs, we were interested in determining the basal level of P-glycoprotein expression in normal tissue. Constitutive high levels of expression could explain primary drug resistance or susceptibility to develop MDR. In addition, the expression pattern of the P-glycoprotein genes might also give a clue to the function of the P-glycoprotein in normal cellular metabolism. Our previous analysis of Chinese hamster ovary and lung cell lines showed very low levels of P-glycoprotein gene expression [8,9]. We have therefore used a sensitive and quantitative method, RNase protection, to determine the levels of P-glycoprotein mRNA [10] in hamster tissues.

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2. MATERIALS AND METHODS

2.1. *RNA isolation and quantitative RNase mapping*

RNA was isolated by the LiCl-urea method [11]. RNA samples were quantitated and checked for integrity by electrophoresis through 0.8% agarose gels. 10 μg of total RNA of each organ and cell line was hybridized with RNA probes under standard conditions [10] and treated with RNase A. The pro-

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00145793/88/$3.50

tected fragments were visualized by electrophoresis through denaturing acrylamide gels followed by autoradiography.

2.2. *RNA probes*

The plasmid CP22s contains a *Xho*I-*Bam*HI fragment of 370 nucleotides (nt) from the 3′-coding region of a hamster P-glycoprotein cDNA cp22 [8] subcloned between the *Bam*HI and *Hind*III sites of pGEM2 (Promega Biotech). Since the vector used contains both an SP6 and T_7 RNA polymerase promotor, RNA probes for the sense and anti-sense strand could be synthesized from one plasmid. cRNA was synthesized from linear templates according to [10]. For sense strand synthesis, the plasmid was linearized with *Pvu*II and transcribed with SP6 RNA polymerase. The anti-sense strand was made using an *Eco*RI linearized template and T_7 RNA polymerase. Since both probes contain transcribed plasmid vector sequences at their 5′- and 3′-end, a distinction can be made between non-digested probe and probe protected by cellular RNA.

3. RESULTS AND DISCUSSION

The P-glycoprotein gene is part of a gene family, with at least three transcribed members in hamster and two in man [9,12–14]. Sequence analysis of cDNA clones shows that the different mRNAs are conserved near the 3′-end of the coding sequence [12]. We have used a cDNA fragment from this conserved region as probe in our assay. The probe, CP22s, corresponds to the transmembrane segment 6a and the nucleotide binding site (positions 755–1125 [12]) of the hamster P-glycoprotein gene originally designated gene class 2C [11] and recently renamed *pgp*1 [12]. *pgp*1 is the homologue of the human *mdr*1 gene. The similarity with the hamster equivalent of the human *mdr*3 gene [13] is unknown.

Total RNA was isolated from Chinese hamster oesophagus, heart, uterus, liver, striated muscle, adrenal gland, kidney, lung, brain, bone marrow, testis, spleen, bladder and ovary. We have also used the Chinese hamster cell lines Aux BI (ovary), CHRC5 (MDR ovary) and A3 (fibroblast). To even out individual variation in P-glycoprotein expression between animals we have pooled organs from six different animals and to avoid polymorphism between the different animals, an inbred Chinese hamster strain was used.

An example of the RNase protection assay is shown in fig.1. Lanes A–F show an RNase protection experiment with RNA derived from testis, uterus, the fibroblast cell line A3 and from the ovarium cell line Aux B1 and the MDR cell line derived from it (CHRC5) [15]. For clarity, the

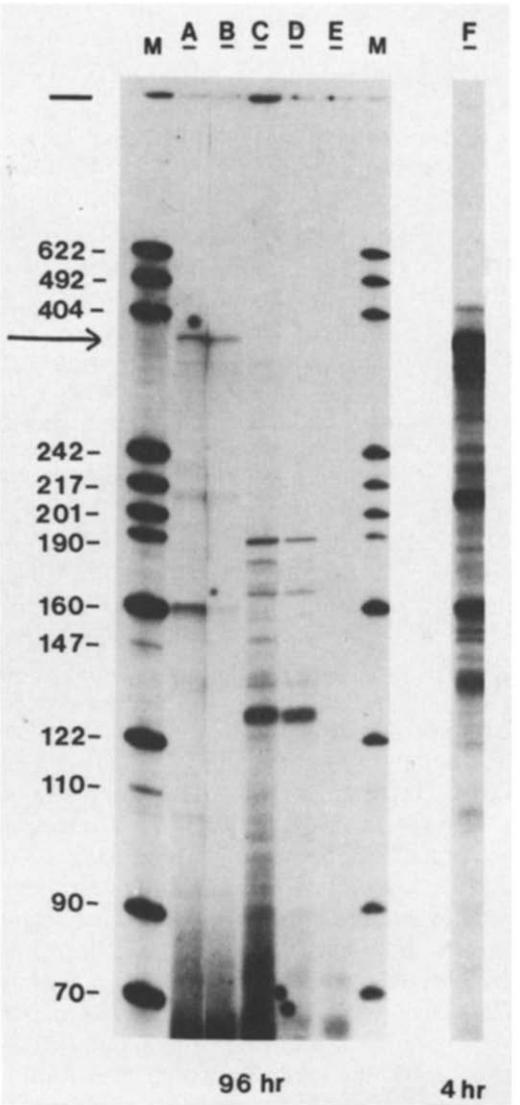

Fig.1. P-glycoprotein mRNA levels in various tissues. Lanes A–E show a 96 h exposure of RNase protection of Aux BI (lane A), the cell line A3 (B), testis (C), uterus (D) and tRNA (E) respectively hybridized with an anti-sense probe. For clarity, the lane corresponding to CHRC5 RNA has been removed from the left panel and a 4 h exposure is shown in lane F. As size marker a radioactively labelled *Msp*I digest of pAT153 was used (lane M). The position of the protected fragment corresponding to the *pgp*1 transcript is indicated by an arrow. For each lane 10 μg of RNA was used.

CHRC5 RNA lane (F) was exposed for only 4 h, whereas the other lanes were exposed for 96 h. The Aux BI (A), A3 (B) and CHRC5 RNA (F) lanes show a band of the expected size (370 nt, indicated

by the arrow); in addition, two other major fragment are visible (210, 160 nt). These additional bands most likely reflect minor differences between the different P-glycoprotein mRNA species (see below). None of these bands are detected after hybridization with tRNA (lane E) and they are thus specific for P-glycoprotein mRNA. Hybridization or RNase digestion at other temperatures or using other RNase A concentrations did not significantly alter the ratio of the expected protected fragments and these additional bands. As a control, all hybridizations were also performed using the sense probe and as expected, no fragments were protected (not shown). This shows that our assay is completely specific and that the hamster P-glycoprotein genes are only transcribed to a significant extent in one direction.

RNA from the Chinese hamster tissues yields another set of fragments. As examples, the signals obtained with testis and uterus RNA are shown in fig.1, lanes C and D. Since the same set of fragments is detected in all 14 tissues, we conclude that the difference in pattern detected between the cell lines and Chinese hamster tissues is due to polymorphic variation between the Chinese hamster strain and the cell lines used. No fragments are detected in any of the tissues by the sense probe. The result of our analysis is summarized in table 1. P-glycoprotein mRNA levels are expressed using the CHO cell line Aux BI as an arbitrary standard. The difference in P-glycoprotein expression between Aux BI and CHRC5 is in agreement with our previous results [8].

The method used here to measure P-glycoprotein gene expression is more sensitive and specific than RNA blot hybridization, but it may underestimate expression of the P-glycoprotein gene family for three reasons: (i) the *pgp*1 probe used will not yield protected fragments of 370 nt with transcripts from the *pgp*2 and *pgp*3 genes. The *pgp*2 transcripts in Aux BI contain a stretch of 160 nt in which only 4 single bases mismatching *pgp*2 are present (positions 973–1125 [12]), flanked by a 3 nt mismatch. Since single basepair mismatches are not easily detected by RNase protection, the 160 nt fragment detected in Aux BI, A3 and CHRC5 RNA most likely reflects the *pgp*2 transcript. The *pgp*3 sequence is not yet available, but in view of the divergence between the human *mdr*1 and *mdr*3 sequence [13], the probe used might not detect the *pgp*3 transcripts. (ii) We have recently shown that at least one of the human *mdr* genes, *mdr*3, which is equivalent to *pgp*3 is alternatively spliced [13]. Alternative splicing of *pgp*1 and *pgp*2 may lead to an underestimation of the expression levels of these genes. (iii) There could be differences between the *pgp*1 gene in the CHO cell line from which the probe is derived and the inbred strain of Chinese hamsters from which the tissue RNAs were prepared. This seems the most likely explanation for the absence of the 370 nt fragment after hybridization with the tissue RNAs, whereas this fragment is detected in the cell line RNAs. Hence, the high levels in table 1 are certainly significant, but low levels may be due to the limitations of the probe used.

Table 1

Tissue/cell line	Relative expression
Adrenal gland	+
Bladder	+
Bone marrow	+
Heart	+
Kidney	+
Liver	+
Spleen	+
Striated muscle	+
A3	+ +
Aux BI	+ +
Brain	+ +
Lung	+ +
Ovary	+ +
Oesophagus	+ + +
Testis	+ + +
Uterus	+ + +
CHRC5	+ + + + +

Expression of the P-glycoprotein gene in Chinese hamster organs and cell lines. The P-glycoprotein mRNA levels are expressed relative to the level in the CHO cell line Aux BI. Expression is measured as radioactivity in the bands > 100 nt in gels as shown in fig.1. Expression levels were determined by comparing band intensities on different autoradiographic exposures with a dilution series of a RNase protection experiment. +, <0.1; + +, 0.1–1; + + +, 1–10; + + + + +, >100

The results in table 1 provide no clue to the nature of the normal function of P-glycoproteins or their contribution to primary drug resistance of mammalian tumors. High expression is found in testis, oesophagus and uterus. These organs are not derived from a common embryonic structure,

nor do they share an abundant common cell type. Testis and uterus also do not yield tumors that are particularly drug resistant. More information may come from in situ hybridization to resolve P-glycoprotein mRNA levels of individual cells in each of these tissues.

Notwithstanding these limitations it is of interest to compare our results with those of Fojo et al. [16], who recently measured *mdr*1 mRNA levels in human tissues by a slot blot hybridization procedure. In human tissues, the authors find the highest levels in the adrenal gland, somewhat lower levels in kidney, colon, liver, lung, jejunum and rectum; even lower levels in brain and prostate; and the lowest levels in skin, subcutaneous tissue, skeletal muscle, heart, spleen, bone marrow, lymphocytes, oesophagus, stomach, uterus, renal cortex and spinal cord. The most remarkable differences are in the mRNA content of liver, adrenal gland (high in man, low in hamster) and oesophagus (low in man, high in hamster). We have verified that the high levels in human adrenal gland and liver are indeed due to *mdr*1 expression and not to the expression of the *mdr*3 gene, which may have been missed in the hamster experiments (Lincke, C., Van der Bliek, A. and Borst, P., unpublished). Moreover, Fojo et al. [16] detect a low *mdr*1 mRNA level in the rat adrenal gland with the probe that gives high levels in human adrenals. This suggests that substantial species differences exist in the tissue specificity of P-glycoprotein gene expression.

Acknowledgements: This work was supported in part by grant NKI 84-20 of the Queen Wilhelmina Fund to P.B. F.B. was supported by the collaborative project on molecular genetics of the University of Amsterdam and the Netherlands Cancer Institute.

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