Increased expression of beta 2-microglobulin in multidrug-resistant tumour cells

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The rat monoclonal antibody LMR-12 was shown earlier to react with a plasma membrane protein, upregulated in multidrug-resistant cell lines. In this study, we observed distinct LMR-12 staining in 36 out of 55 non-drug-selected tumour cell lines, including melanomas, renal cell-, colon- and lung carcinomas, whereas in other tumour types, such as leukaemia and ovarian cancer, LMR-12 staining was generally low or absent. The cDNA encoding the LMR-12 antigen was isolated from a library of the multidrug-resistant human fibrosarcoma cell line HT1080/DR4 by expression cloning in MOP8 cells. Sequence analysis showed that the LMR-12 antigen is identical to the major histocompatibility complex class I molecule beta 2-microglobulin (β2-m). The LMR-12/β2-m staining results were confirmed by mRNA microarray data from an independent National Cancer Institute study, as well as by newly obtained reverse transcriptase polymerase chain reaction data. Further analysis of the microarray data showed that β2-m levels closely reflected levels of major histocompatibility complex class I heavy chains and the transporter associated with antigen processing. Since the ABC transporter associated with antigen processing was previously shown to contribute to multidrug-resistance, it may very well be that the observed LMR-12/β2-m levels are secondary to (elevated) levels of the transporter associated with antigen processing. A perspective arising from the present study is that drug resistant tumour cells may, by having elevated levels of major histocompatibility complex related molecules, be particular good candidates for alternative therapeutic therapies, such as cytotoxic T cell mediated immune-therapies.

Keywords: LMR-12; multidrug resistance; expression cloning; beta 2-microglobulin; MHC class I; TAP
described previously (Wu et al., 1992). The panel includes cell lines derived from cancers of the colon, kidney, lung, breast, ovary, and brain, as well as melanoma and leukaemia. All other cell lines used have been described in the literature and are mentioned in Scheffer et al. (2000). The cell lines included the small cell lung carcinoma cell line GLC4 and its adriamycin-resistant (MRPl positive) subline GLC4/ADR; the leukaemia cell lines HL60 and the adriamycin-resistant (MRPl positive) HL60/ADR subline; the fibrosarcoma cell line HT1080 and its daunorubicin resistant subline HT1080/DR4. The polyoma transformed NIH3T3 mouse fibroblast cell line MOP8 (Muller et al., 1984) was used for transfection experiments.

The resistant sublines were routinely cultured in the presence of cytotoxic drug until 3–10 days before the experiments. The cell lines were cultured in RPMI-1640 or Dulbecco’s modified essential medium (DMEM; Gibco Europe, Paisley, Scotland) with 10% heat-inactivated foetal calf serum (Gibco Europe). The panel of 34 human tumours of different histogenetic origins included (snap frozen) tumours of the intestine, stomach, testis, prostate, lung, pancreas, bladder, adrenal gland, cervix, neurologic tissue, breast, ovary, kidney and melanoma.

Immunohistochemistry

Cytoxentrifuge preparations of tumour cell lines or frozen sections of tumour samples were air-dried overnight and fixed for 7 min with 100% acetone for 7 min and stained with LMR-12 (1:500) to detect transiently expressed protein as described above. A colony containing the cDNA coding for the LMR-12 antigen was isolated by screening progressively smaller pools of bacterial colonies.

cDNA sequence analysis

The LMR-12 cDNA clone was sequenced using the dyelexo Terminator Cycle Sequencing Kit on an automated 373A DNA sequencer (Applied Biosystems Benelux B.V., Maarsen, The Netherlands). Sequencing was performed in both orientations to confirm the nucleotide sequence. The data were collected and analysed using 373A computer software.

RNA isolation and RT – PCR

Total RNA was isolated using the RNasey kit and the QIAvac vacuum manifold (Qiagen, Valencia, CA, USA) per manufacturer’s protocol. Total RNA samples were quantitated using the fluoroscent Ribogreen RNA quantitation reagent kit (Molecular probes) and RNA was stored at −70°C until use for RT – PCR. The RT – PCR reactions were measured with the ABI Prism 7700 Sequence Detection System using TaqMan one-step RT – PCR SYBR green PCR master mix in 50 μl reactions using 5 ng total RNA per reaction. Primers for β2-m were designed with Primer Express software (PE Biosystems, Foster City, CA, USA; forward primer: GCCATTCAGCGTACTCCAAAG, reverse primer: CAACITCAAGTTCGATGGTYG). GAPDH was used as an endogenous control (forward primer: GAAGGTGAAGGTTGCAAAG, reverse primer: GAAGGTGAAGGTCCGTCGTC). Primers concentrations for β2-m and GAPDH were 600 and 100 nM respectively. Thermocycler parameters were 30 min at 48°C, 10 min at 95°C and 40 PCR cycles of 15 s at 95°C and 1 min at 60°C. All RNA samples were tested in triplicate PCR reactions. Data was analysed using the comparative CT method (Perkin Elmer, user bulletin #2) and β2-m was normalised to a calibrator cell line (HCC-2998).

Correlative and COMPARE analysis

Using SPSS 9.0 statistical software, two-tailed Pearson correlation coefficients (PCC) were calculated between (LMR-12) immunohistochemical staining data, microarray data from Ross et al. (2000) and Scherf et al. (2000) (available at the NCI Developmental Therapeutics Program website (http://dtp.nci.nih.gov)), and β2-m mRNA data from the RT – PCR. The COMPARE program of the NCI Developmental Therapeutics Program was used to examine the correlation between the LMR-12 staining results and sensitivity for compounds tested, and with basal patterns of gene expression in the 55 cell lines measured by cDNA microarray.

Tumour samples

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RESULTS

Frequent LMR-12 staining in tumour cell lines

As reported previously (Flens et al, 1997), LMR-12 staining was observed in a broad panel of MDR cell lines, with higher levels in the resistant sublines. Among these cell lines are the paired parental and resistant cell lines GLC4 and GLC4/ADR, the HL60 and HL60/ADR and the HT1080 and HT1080/DR4 cell line. To further study the prevalence of the LMR-12 antigen, a panel of drug-unselected human tumour cell lines of the US National Cancer Institute’s anticancer drug screening program, cytosol of these cell lines were examined for LMR-12 expression. In 36 out of 55 tumour cell lines of the panel LMR-12 staining was

Table 1  Staining indices for MDR1 P-gp, MRPI, MVP, LMR-12/β2-m, relative LMR-12 staining values and β2-m mRNA values in the NCI panel of human cancer cell lines

| Cell line      | Tumour type | MDR1 P-gp | MRPI | MVP | LMR-12/β2-m rel. LMR-12 staining | β2-m microarray | rel. β2-m RT-PCR |
|---------------|-------------|-----------|------|-----|---------------------------------|----------------|------------------|
| MALME-3M      | Melanoma    | ○         | ●    | ●   | 2.04                             | 0.07           | −0.06            |
| M14           | Melanoma    | ○         | ●    | ○   | 1.04                             | 0.21           | 1.53             |
| SK-MEL-5      | Melanoma    | ○         | ●    | ●   | 0.44                             | −0.12          | −1.08            |
| SK-MEL-28     | Melanoma    | ○         | ●    | ○   | 0.64                             | −0.09          | −2.97            |
| UACC-2457     | Melanoma    | ○         | ●    | ●   | 0.24                             | −0.19          | −1.82            |
| M19-MEL       | Melanoma    | ○         | ●    | ●   | ND                               | ND            | ND               |
| SK-MEL-2      | Melanoma    | ○         | ●    | ○   | −0.06                            | 0.22           | 2.12             |
| UACC-62       | Melanoma    | ○         | ●    | ○   | −0.56                            | 0.06           | 4.83             |
| LOX           | Melanoma    | ○         | ●    | ○   | −0.91                            | −0.18          | −1.68            |
| TK-10         | Renal       | ○         | ●    | ●   | 2.04                             | 0.17           | −3.04            |
| RXF-393       | Renal       | ○         | ●    | ●   | 0.94                             | 0.40           | 3.18             |
| ACHN          | Renal       | ●         | ●    | ●   | −1.11                            | 0.13           | 2.49             |
| RXF-631       | Renal       | ○         | ●    | ○   | ND                               | ND            | ND               |
| SN12          | Renal       | ○         | ●    | ●   | −0.06                            | −0.05          | −2.07            |
| A498          | Renal       | ○         | ND   | ND  | −0.36                            | −0.30          | 0.99             |
| 786-0         | Renal       | ○         | ●    | ○   | −0.96                            | 0.12           | 1.49             |
| COLO-205      | Colon       | ○         | ●    | ●   | 2.04                             | 0.30           | 0.13             |
| HCT-116       | Colon       | ○         | ●    | ●   | 0.94                             | 0.35           | 2.54             |
| HT29          | Colon       | ○         | ●    | ●   | ND                               | ND            | ND               |
| KM2012        | Colon       | ○         | ●    | ●   | −0.36                            | −0.24          | −1.95            |
| SW-620        | Colon       | ○         | ●    | ●   | −0.96                            | −0.62          | −2.78            |
| KM12          | Colon       | ○         | ●    | ●   | −0.96                            | −0.62          | −2.78            |
| SCC-2998      | Colon       | ○         | ●    | ●   | ND                               | ND            | ND               |
| HCT-15        | Colon       | ○         | ●    | ●   | ND                               | ND            | ND               |
| DLD-1         | Colon       | ○         | ●    | ●   | ND                               | ND            | ND               |
| HOP-62        | Lung        | ○         | ●    | ●   | 2.04                             | −0.11          | −1.86            |
| NCI-H226      | Lung        | ○         | ●    | ●   | 2.04                             | 0.48           | 6.29             |
| DSM-114       | SCLC        | ○         | ●    | ●   | ND                               | ND            | ND               |
| HOP-92        | Lung        | ○         | ●    | ●   | 0.74                             | 0.42           | −0.29            |
| LXLF-529L     | Lung        | ○         | ●    | ●   | ND                               | ND            | ND               |
| BOVX          | Lung        | ○         | ●    | ●   | ND                               | ND            | ND               |
| NCI-H23       | Lung        | ○         | ●    | ●   | −0.71                            | −0.26          | −1.16            |
| HOP-18        | Lung        | ○         | ●    | ●   | ND                               | ND            | ND               |
| AS49          | Lung        | ○         | ●    | ●   | −0.96                            | −0.35          | −3.06            |
| NCI-H522      | Lung        | ○         | ●    | ●   | ND                               | ND            | ND               |
| DSM-273       | SCLC        | ○         | ●    | ●   | ND                               | ND            | ND               |
| SNB-75        | CNS         | ○         | ●    | ●   | 1.29                             | 0.48           | 2.40             |
| SF-539        | CNS         | ○         | ●    | ●   | 1.89                             | 0.66           | 3.29             |
| SF-295        | CNS         | ○         | ●    | ●   | 0.24                             | 0.25           | 0.42             |
| XF-498        | CNS         | ○         | ●    | ●   | ND                               | ND            | ND               |
| SF-268        | CNS         | ○         | ●    | ●   | ND                               | ND            | ND               |
| SNB-19        | CNS         | ○         | ●    | ●   | ND                               | ND            | ND               |
| SNB-78        | CNS         | ○         | ●    | ●   | ND                               | ND            | ND               |
| RPMI 8260     | Leukemia    | ○         | ●    | ●   | 1.74                             | 0.59           | 4.85             |
| HL-60         | Leukemia    | ○         | ●    | ●   | −0.36                            | 0.20           | −0.98            |
| CEM           | Leukemia    | ○         | ●    | ●   | −0.96                            | 0.14           | 2.69             |
| K-562         | Leukemia    | ○         | ●    | ●   | −0.96                            | 0.14           | −4.02            |
| SR            | Leukemia    | ○         | ●    | ●   | −0.96                            | 0.72           | 2.69             |
| MOLT-4        | Leukemia    | ○         | ●    | ●   | −0.96                            | −0.02          | −3.81            |
| OVCAR-5       | Ovary       | ○         | ●    | ●   | 0.04                             | 0.15           | 2.49             |
| OVCAR-4       | Ovary       | ○         | ●    | ●   | −0.86                            | −0.02          | −3.03            |
| SK-OV-3       | Ovary       | ○         | ●    | ●   | −0.71                            | 0.10           | 0.63             |
| OVCAR-8       | Ovary       | ○         | ●    | ●   | −0.96                            | −1.00          | −0.83            |
| IGROV1        | Ovary       | ○         | ●    | ●   | −0.96                            | −0.20          | −1.62            |
| OVCAR-3       | Ovary       | ○         | ●    | ●   | −0.96                            | −0.34          | −3.47            |

The staining indices of MDR1 P-gp, MRPI and MVP are given for comparison (MDR1 P-gp adapted from Wu et al (1992), MRPI and MVP adapted from Izquierdo et al (1996a)). The staining indices were calculated as the product of the fraction of positive cells and staining intensity, and presented as follows: 0 = ○, 0.1–0.5 = ●, 0.5–1.0 = ●●, 1.0–2.0 = ●●●, 2.0–3.0 = ●●●●. ND = not determined. The β2-m mRNA data are adapted from Ross et al (2000) and Scherf et al (2000). The RT-PCR data were normalised to a calibrator cell line (HCC-2998). The relative expression data are equivalent to ‘mean graphs’; expression results were averaged and each value is expressed as a function of the average value.
found (Table 1 and Figure 1). In the table the staining data of MDR1 P-gp, MRP1 and MVP are given for comparison (MDR1 P-gp adapted from Wu et al (1992), MRP1 and MVP adapted from Izquierdo et al (1996a)). LMR-12 positive staining was particularly evident in distinct tumour types i.e. melanomas (seven out of nine cell lines), renal cell carcinomas (six out of seven), colon carcinomas (six out of nine cell lines) and lung carcinomas (six out of 11 cell lines). In leukemia and ovarian cancers generally low expression was observed. LMR-12 staining was frequently accompanied by MVP-, MRP1- and/or MDR1 P-gp staining, but only with MVP staining a significant correlation was observed ($r =0.515$, $P <0.001$).

**β2-m is the LMR-12 antigen**

As reported previously (Flens et al, 1997), Western blot experiments did not give information on the nature of the cognate antigen of the LMR-12 Mab, not even when 125Iodine-labelled LMR-12 was used in the experiments. Also, immunoprecipitation experiments did not reveal the antigen. Therefore, the expression cloning system that had successfully been used to identify the nature of the LRP-56 Mab (Scheffer et al, 1995) was also used here. MOP8 cells were transiently transfected with pCDM8 vector, containing cDNA inserts of the MDR fibrosarcoma cell line HT1080/DR4 and screened for LMR-12 immunoreactivity. In the primary screening, MOP8 cells transfected with cDNAs from one of the sublibraries showed one single LMR-12 immunoreactive MOP8 cell among approximately $3 \times 10^6$ cells. The coding cDNA clone was isolated from the sublibrary after several cycles of screening of MOP8 cells transfected with cDNA’s from progressively smaller pools of bacterial colonies for transiently expressed antigen.

Sequence analysis of both ends of the LMR-12 cDNA, and database searches with the obtained sequence information revealed that the LMR-12 antigen is the human major histocompatibility complex (MHC) protein β2-m.

**LMR-12/β2-m staining data correlates with mRNA data**

To confirm the expression data on β2-m in the cell line panel, the correlation between the LMR-12/β2-m staining data and microarray data on mRNA levels for β2-m was examined. Ross et al (2000) and Scherf et al (2000) have reported microarray data on approximately 8000 genes in the cell lines of the NCI panel, including β2-m. These data are available via http://dtp.nci.nih.gov. From a total of 45 cell lines, both protein and mRNA data could be compared. The analysis showed a moderate, but highly significant correlation (Tables 1 and 2 and Figure 2) for β2-m protein and mRNA data in these studies.

To further explore the correlation between LMR-12/β2-m protein and mRNA data, a light-cycle RT–PCR was performed.

![Figure 1](https://example.com/figure1.png)

**Figure 1** LMR-12 staining of cytospins of cell lines in the NCI cell line panel (see also Table 1); COLO-205 (colon, strongly positive), SN12 (renal, weakly positive) and SR (leukaemia, negative). Colour development was with 0.02% (w v$^{-1}$) amino-ethyl-carbazole and 0.02% (v v$^{-1}$) H$_2$O$_2$.

**Table 2** Pearson correlation coefficients for HLA-A, HLA-B, β2-m and TAP1 mRNA microarray analyses, and β2-m RT–PCR and LMR-12/β2-m staining results

|            | HLA-A array | HLA-B array | β2-m array | β2-m RT–PCR | LMR-12 staining | TAP1 array |
|------------|-------------|-------------|------------|-------------|----------------|------------|
| HLA-A array| PCC 1.000   | 0.796       | 0.402      | 0.435       | 0.494          | 0.614      |
| p          | <0.001      | 0.001       | 0.003      | 0.001       | 0.001          | <0.001     |
| n          | 60          | 60          | 60         | 45          | 45             | 59         |
| HLA-B array| PCC 0.796   | 1.000       | 0.339      | 0.389       | 0.434          | 0.688      |
| p          | <0.001      | 0.008       | 0.008      | 0.003       | 0.001          | <0.001     |
| n          | 60          | 60          | 60         | 45          | 45             | 59         |
| β2-m array | PCC 0.402   | 0.339       | 1.000      | 0.606       | 0.445          | 0.441      |
| p          | 0.001       | 0.008       | <0.001     | 0.002       | 0.002          | <0.001     |
| n          | 60          | 60          | 60         | 45          | 45             | 59         |
| β2-m RT–PCR| PCC 0.435   | 0.389       | 0.606      | 1.000       | 0.318          | 0.398      |
| p          | 0.003       | 0.008       | <0.001     | 0.003       | 0.033          | 0.007      |
| n          | 45          | 45          | 45         | 45          | 45             | 44         |
| LMR-12 staining | PCC 0.494 | 0.434 | 0.445 | 0.318 | 1.000 | 0.316 |
| p | 0.001 | 0.003 | 0.002 | 0.033 | 0.037 | 0.027 |
| n | 45 | 45 | 45 | 45 | 45 | 44 |
| TAP1 array | PCC 0.614   | 0.688       | 0.441      | 0.298       | 0.316          | 1.000      |
| p | <0.001 | <0.001 | <0.001 | 0.007 | 0.037 | 0.027 |
| n | 59 | 59 | 59 | 44 | 44 | 59 |

PCC=Pearson correlation coefficient; p=significance, significant when $P <0.05$; n=number of samples.
on RNA isolated from the NCI cell lines. These RT–PCR values of the cell lines, normalised to a calibrator cell line (HCC-2998), also showed a moderate, but significant correlation with the LMR-12/\(\beta_2\)-m staining data (Tables 1 and 2 and Figure 2). Correlation between RT–PCR data and microarray data was highly significant (Tables 1 and 2 and Figure 2).

To further examine the correlation of the LMR-12/\(\beta_2\)-m data with MHC class I associated molecules, statistical analyses were done on microarray data available for these molecules. (Highly) significant correlations were observed for both LMR-12/\(\beta_2\)-m staining data and \(\beta_2\)-m mRNA data with microarray data for the MHC class I heavy chains HLA-A and HLA-B, as well as with

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**Figure 2** Meangraphs of LMR-12 staining, \(\beta_2\)-m RT–PCR data and \(\beta_2\)-m microarray data in 45 cell lines of the anticancer drug screen. The RT–PCR data were normalised to a calibrator cell line (HCC-2998). The \(\beta_2\)-m microarray data are available at http://dtp.nci.nih.gov. The meangraphs were made from relative expression data; expression results were averaged and each value was expressed as a function of the average value (see Table 1). Correlations calculated from these data are presented in Table 2.

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the ABC transporter associated with antigen processing TAP1 (Table 2).

LMR-12/β2-m staining correlates with W6/32 MHC class I staining

To further confirm the association of LMR-12/β2-m staining with MHC class I heavy chain, we FACS analysed the staining of LMR-12/β2-m, the anti-MHC class I Mab W6/32 and a polyclonal anti β2-m antiserum in paired parental and MDR cell lines. The results observed with the latter two reagents were highly comparable to the LMR-12 results. Elevated staining of all three antisera was observed in the GLC4/ADR cells and in the HL60/ADR cells compared to their parental cell lines (Figure 3).

In a panel of 34 human tumours of different histogenetic origin, clear LMR-12/β2-m staining was observed in ~90% of the tumour samples, while the surrounding normal tissue always stained positive. The staining pattern of LMR-12/β2-m was closely paralleled by W6/32 staining (Figure 4).

COMPARE analysis

When the LMR-12/β2-m staining values for the tumour cell lines were utilised as a ‘seed’ pattern for the COMPARE program, no correlations greater than 0.46 were observed for anticancer agents tested. Extensions of these analyses to the whole synthetic database showed no correlations greater than 0.6 and no clear chemical structural theme could be observed in the top ranked compounds. Application of the COMPARE analysis to the molecular targets database yielded MVP as the second ranked pattern. The first ranked pattern was the BCL2-antagonist of cell death (BAD). While these were the best correlations observed, they were not particularly strong, with Pearson correlation coefficients (PCC) in the 0.3 – 0.4 range. MRP1 also showed up in this analysis, ranked at position 17.
with a PCC of 0.2. The top reverse COMPARE hit was glutathione S-transferase (GST) π with a PCC of 0.454.

Comparison to the microarray database yielded several unfamiliar genes in the top 10, but also two MHC class I molecules, F and A, at position 11 and 12, respectively, and with PCCs of around 0.5. Also, \( \beta_2 \)-m showed up in the analysis, at position 26, with a PCC of 0.47.

**DISCUSSION**

Staining with the rat antibody LMR-12 was reported to be upregulated in a broad panel of MDR cell lines (Flens et al, 1997). In this study we found that the LMR-12 antigen is also present in 36 out of 55 samples of a panel of drug-unselected primary tumour cells. Staining was particularly evident in distinct tumour cell types that are in general considered as refractory to chemotherapy, i.e. melanomas, renal cell, colon- and lung carcinomas. Tumour cells considered more sensitive to chemotherapy, such as leukaemia and ovarian carcinoma showed only low staining levels. LMR-12 staining was never exclusive and correlated significantly with MVP staining, but not with MRP1 or MDR1 P-gp staining. Collectively, these results suggested the potential relevance of the cognate antigen in resistance of tumour cells to cytostatic drug exposure.

Considering that drug resistance continues to represent a major obstacle in clinical cancer treatments, we set out to identify the antigen recognised by the LMR-12 Mab. As conventional Western blot and immunoprecipitation techniques had proven unsuccessful to give information on the identity of the antigen of this Mab, we used an eukaryotic expression cloning procedure. The LMR-12 Mab was used as a probe to detect transiently expressed protein in transfected MOP8 cells and to isolate the cDNA encoding the antigen.

Sequence analysis and database searches showed that the LMR-12 antigen has no homology with ABC transporter proteins involved in MDR. Instead, the protein turned out to be identical to the human MHC class I molecule \( \beta_2 \)-m. The M1, 12 000 \( \beta_2 \)-m associates with MHC class I heavy chains to form class I human leukocyte antigen (HLA) complexes.

When comparing our LMR-12/ \( \beta_2 \)-m staining data in the NCI cell line panel to independent, previously reported \( \beta_2 \)-m mRNA microarray data and to newly obtained RT–PCR mRNA data, we observed moderate, but significant correlations between protein and mRNA data. The RT–PCR data correlated highly significantly with the microarray data. As yet, only a few reports have been published on the correlation between mRNA and protein levels in cells and tissues, whereas none has correlated microarray data with RT–PCR data. In our observations, Anderson and Seilhamer (1997) found a correlation coefficient of 0.48 for mRNA levels and proteins expressed in human liver. These, acceptable, but non-perfect correlations indicate that post-transcriptional regulation of gene expression is a frequent phenomenon in higher organisms and very likely holds true for \( \beta_2 \)-m as well. Furthermore, \( \beta_2 \)-m transcript levels appeared to vary considerably in individual cell lines, making \( \beta_2 \)-m a poor candidate for standardising mRNA levels as detected by e.g. RT–PCR. Although similar findings were already reported by Finkle et al. (1993), still many researchers erroneously choose \( \beta_2 \)-m as a standard housekeeping gene.

The observed significant correlation between microarray data and newly obtained data as mRNA data obtained by RT–PCR, indicates that publicly accessible databases containing microarray data for large numbers of genes can be an important valid information source for researchers.

Further access of the microarray database to correlate our \( \beta_2 \)-m results to reported microarray data on MHC related molecules showed that both \( \beta_2 \)-m staining and mRNA data (highly) significantly correlated with mRNA data for MHC class I heavy chains and TAP1. The connection of the LMR-12/ \( \beta_2 \)-m data with MHC class I heavy chains was also confirmed by staining results with the W6/32 Mab both in FACS experiments and immunohistochemical stainings of human tumour sections. Also in the COMPARE analysis to the microarray database MHC class I molecules turned up at high positions in the list. When compared to molecular targets, interesting molecules such as GST π, BAD and the MDR marker MVP turned up, but no high correlations were observed for anticancer agents tested.

As \( \beta_2 \)-m itself has no characteristics of a transporter protein, it seems very unlikely that the protein itself plays a causal role in the MDR phenotype. Still, upregulation of MHC related molecules in MDR cells was already observed in previous studies. Iguchi et al. (2001) noted upregulation of MHC class I molecules, including \( \beta_2 \)-m, in gastric- and colon cancer cell lines after treatment with the plant polysaccharide PSK, which was examined for anti-tumour effects. Furthermore, in a study to examine the possible contribution of the ABC transporter associated with antigen processing (TAP) to MDR, we showed that in our panel of MDR cell lines elevated TAP levels were paralleled by elevated levels of MHC class I molecules (Izquierdo et al, 1996b). The TAP molecule plays a major role in MHC class I-restricted antigen presentation by mediating peptide translocation over the endoplasmic reticulum membrane.

In the same study a moderate contribution of TAP to MDR was demonstrated by transfection of the TAP genes into TAP-deficient lymphoblastoid T2 cells, resulting in low level resistance to etoposide, vincristine and doxorubicin. Therefore, it seems valid to conclude that the observed elevated levels of \( \beta_2 \)-m in this study most likely are the consequence of upregulation of TAP. Recently, the antigen of LMR-42, another MDR-related Mab originating from the same study as the LMR-12 Mab (Flens et al, 1997), was identified as the endothelial cell protein C receptor (EPCR). This type 1 transmembrane glycoprotein is also a member of the MHC superfamily (Scheffer et al, 2002). In contrast to these findings of upregulated MHC related molecules, many studies have reported on downregulation or loss of expression of MHC class I related molecules in malignant cells (Kakkamakis et al, 1995; Korkolopoulou et al, 1996; Keskinen et al, 1997; Gudmundsdottir et al, 2000; Kamarashe et al, 2001; Palmisano et al, 2001). Ogretmen et al. (1998) even reported the loss or decreased expression of \( \beta_2 \)-m in drug-resistant sublines of MCF-7 and HL60 cells, controlled by post-transcriptional mechanisms. These authors also reported that the partial inhibition of \( \beta_2 \)-m by antisense RNA resulted in 2–3-fold decreased sensitivity of MCF-7 cells to doxorubicin and mAMSA. An explanation for these opposing results may be, partially, found in utilising other (sub) lines of cell lines and differences in culture conditions. MHC class I levels may be influenced by differences in culture medium components, and the presence or absence of stimulating cytokines such as interferon gamma (Keskinen et al, 1997). From a different perspective the obtained results may be interesting for therapeutic treatments as well. Drug resistant tumour cells may, by having elevated levels of MHC related molecules, be particular good candidates for alternative therapies, such as cytotoxic T cell mediated immune-therapies.

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REFERENCES

Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM (1999) Biochemical, cellular, and pharmacological aspects of the multidrug transporter. *Ann Rev Pharmacol Toxicol* 39: 361 – 398

Anderson L, Seilhamer J (1997) A comparison of selected mRNA and protein abundances in human liver. *Electrophoresis* 18: 533 – 537

Arufo A, Seeb B (1987) Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. *Proc Natl Acad Sci USA* 84: 8573 – 8577

Barnstable CJ, Bodmer WF, Brown G, Galfre G, Milstein C, Williams AF, Ziegler A (1978) Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens-new tools for genetic analysis. *Cell* 14: 9 – 20

Borst P, Evers R, Koel M, Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* 92: 1295 – 1302

Cole SP, Deeley RG (1997) Analysis of expression of selected mRNA and protein multimers of the multidrug transporter. *J Natl Cancer Inst* 90: 148 – 153

Doyle LA, Yang WD, Abruzzo LV, Krogmann T, Gao YM, Rishi AK, Ross DD (1998) A multidrug resistance transporter from human MCF-7 breast cancer cells (erratum in *PNAS* USA 1999; 96(5):2569). *Proc Natl Acad Sci USA* 95: 15665 – 15670

FinneGAN MC, Goepel JR, Hancock BW, GoyNS MH (1993) Investigation of the expression of housekeeping genes in non-Hodgkin’s lymphoma. *Leuk Lymphoma* 10: 387 – 393

Flems MJ, Scherer GL, van der Valk P, Broxterman HJ, Eijeds EM, Huysmans AC, Izquierdo MA, Scherer RJ (1997) Identification of novel drug resistance-associated proteins by a panel of rat monoclonal antibodies. *Int J Cancer* 73: 249 – 257

Gudmundsdottir I, Gunnlaug Jonasson J, Sigurdsson H, Olafsdottir K, Finnegan MC, Goepel JR, Hancock BW, Goyns MH (1993) Investigation of the expression of housekeeping genes in non-Hodgkin’s lymphoma. *Leuk Lymphoma* 10: 387 – 393

Higgins CF (1992) ABC transporters – from microorganisms to man. *Ann Rev Cell Biol* 8: 67 – 113

Iguchi C, Nio Y, Takeda H, Yamasawa K, Hirahara N, Toga T, Itakura M, Kaklamanis L, Leek R, Koukourakis M, Gatter KC, Harris AL (1995) Loss of antigen-presenting molecules (MHC class I and TAP-1) in metastatic versus primary breast cancer. *Int J Cancer* 60: 500 – 505

Izquierdo MA, Neeefjes JJ, Mathari AE, Flens MJ, Scherer GL, Scherer RJ (1996b) Overexpression of the ABC transporter TAP in multidrug-resistant human cancer cell lines. *Br J Cancer* 74: 1961 – 1967

Izquierdo MA, Shoomaker RH, Flens MJ, Scherer GL, Wu L, Prather TR, Scherer RJ (1996a) Overlapping phenotypes of multidrug resistance among panels of human cancer-cell lines. *Int J Cancer* 65: 230 – 237

Kaklamanis I, Leek R, Koukourakis M, Gatter KC, Harris AL (1995) Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I molecules in metastatic versus primary breast cancer. *Cancer Res* 55: 5191 – 5194

Kamarashev J, Ferrone S, Seifert B, Boni R, Nestle FO, Burg G, Dummer R (2001) TAP1 down-regulation in primary melanoma lesions: an independent marker of poor prognosis. *Int J Cancer* 95: 23 – 28

Kool M, de Haas M, Scherer GL, Scherer RJ, van Eijk MJ, Iuijn JA, Baas F, Borst P (1997) Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res* 57: 3537 – 3547

Korkolopoulou P, Kaklamanis I, Pezzella F, Harris AL, Gatter KC (1996) Loss of antigen-presenting molecules (MHC class I and TAP-1) in lung cancer. *Br J Cancer* 73: 148 – 153

Muller WJ, Naujokas MA, Hassell JA (1984) Isolation of large T antigen-producing mouse cell lines capable of supporting replication of polyoma-virus-plasmid recombinants. *Mol Cell Biol* 4: 2406 – 2412

Ogretmen B, McCauley MD, Safa AR (1998) Molecular mechanisms of loss of beta 2-microglobulin expression in drug-resistant breast cancer sublines and its involvement in drug resistance. *Biochemistry* 37: 11679 – 11691

Palmisano GL, Pistillo MP, Capanni P, Pera C, Nicolò G, Salvi S, Perdelli L, Pascucci G, Ferrara GB (2001) Investigation of HLA class I downregulation in breast cancer by RT–PCR. *Hum Immunol* 62: 133 – 139

Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, Iyer V, Jeffrey SS, van de Rijn M, Waltham M, Parmigenskichov A, Lee JC, Lashkari D, Shalon D, Myers TG, Weinstein JN, Botstein D, Brown PO (2000) Systematic variation in gene expression patterns in human cancer cell lines. *Nature Genet* 24: 227 – 235

Scheffer GL, Flens MJ, Hageman S, Izquierdo MA, Shoomaker RH, Scherer RJ (2002) Expression of the vascular endothelial cell protein C receptor in epithelial tumor cells. *Eur J Cancer* (in press)

Scheffer GL, Kool M, Heijn M, de Haas M, Pijnenborg ACLM, Wijnholds J, van Helvoort A, de Jong MC, Hooijberg JH, Mol CAAM, van der Linden M, van de Rijn P, van der Valk P, Oude Elferink RPI, Borst P, Scherer RJ (2000) Specific detection of multidrug resistance proteins MRP1, MRP2, MRP3, MRPs and MDR3 P-glycoprotein with a panel of monoclonal antibodies. *Cancer Res* 60: 5269 – 5277

Schneider E, Paul D, Ivy P, Cowan KH (1999) Multidrug resistance. *Cancer Chemother Biol Response Modif* 14: 152 – 177

Tan TM, Ivanji P, Hilgert I, Brdicka T, Pla M, Breur B, Flieger M, Ivaskova E, Horejsi V (2001) The epitope recognized by pan-HLA class I-reactive monoclonal antibody W6/32 and its relationship to unusual stability of the HLA-B27/beta2-microglobulin complex. *Immunogenetics* 53: 440 – 446

Wu L, Smythe AM, Stinson SF, Mullendore LA, Monks A, Scudiero DA, Paull KD, Koutsoukous AD, Rubinstein LV, Boyd MR, Shoomaker RH (1992) Multidrug-resistant phenotype of disease-oriented panels of human tumor cell lines used for anticancer drug screening. *Cancer Res* 52: 3029 – 3034