SHORT COMMUNICATION

A monoclonal antibody detecting cell surface epitope on some drug resistant human tumour cell lines

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Antineoplastic agents may be very effective in the first round of combination chemotherapy; however, upon subsequent treatment, many tumours display resistance. In many instances, drug resistance is observed to multiple agents which differ in structure as well as in their mechanism of action. Development of such multidrug resistance (MDR) is a major obstacle in the treatment of a variety of malignancies (Kaye, 1988). Although the altered expression of a number of proteins has been associated with MDR in different model systems, the overexpression of a 150–180 kDa glycoprotein has been the most consistent. This glycoprotein, termed P-glycoprotein, acts as an energy dependent efflux pump to reduce drug accumulation within the cell (Gerlach et al., 1986). While overexpression of P-glycoprotein is clearly responsible for MDR in some cell systems, it is unlikely that P-glycoprotein by itself can account for the plethora of biochemical and genetic changes which occur as a cell adapts to growth in the presence of antineoplastic agents (Kaye, 1988). The isolation of MDR cell lines which do not overexpress P-glycoprotein, and the detection of P-glycoprotein in only a subset of patients with drug-resistant tumours (Goldstein et al., 1989), support a multifactorial model of MDR (Mirska et al., 1987; Danks et al., 1987; Slovák et al., 1988; McGrath et al., 1989).

A number of monoclonal antibodies have been derived with specificity for P-glycoprotein and have proven to be valuable tools in the analysis of the MDR phenotype mediated by P-glycoprotein (Kartner et al., 1985; Schepet et al., 1988; Hamada & Tsuruo, 1988a, b; Thiebaut et al., 1987).

In the present study, we report the derivation of a monoclonal antibody against a MDR human ovarian carcinoma cell line, A2780-AD (AD) (Rogan et al., 1984), which recognises a cell surface antigen whose association with drug resistance appears independent of P-glycoprotein.

A 6–8-week-old female Balb/c mouse was immunised with four i.p. injections, each consisting of 10×10⁶ viable AD cells, on days 1, 7, 17 and 35 days. Four days following an i.v. injection of 5×10⁶ AD cells on day 59, the immune spleen cells were fused with P3.NS1/Ag4.1 (NS-1) myeloma cells using a 50% (w/v) polyethylene glycol solution (Kennett, 1979). Hybrids were selected in hypoxanthine, aminopterin and thymidine-containing medium and at 10–14 days post-fusion, supernatants from wells containing macroscopically visible hybridomas were tested for specific antibodies using an indirect enzyme-linked immunosorbent assay (ELISA) with AD cells and the corresponding drug-sensitive A2780-9S (9S) cells (Glassy & Surh, 1985; Mirski et al., 1987). The criteria used to determine specific reactivity were:

(i) a ratio of absorbance values (A₄₀₀ nm AD:A₄₀₀ nm 9S) greater than 3;
(ii) absorbance values on 9S cells similar to negative control values; and
(iii) consistent ELISA reactivity after multiple passages in culture. Five hybridomas met these criteria and were cloned by limiting dilution (Kozioł et al., 1987). Hybridomas were cryopreserved and supernatants were collected and frozen at −20°C. One, designated MAB 7.4.1, was selected for further study; the antibody secreted by this hybridoma was IgG₁ (Boehringer-Mannheim isotype kit).

The subcellular location of the epitope on AD cells recognised by MAB 7.4.1 was determined by indirect immunofluorescence. By fluorescence microscopy, MAB 7.4.1 labelled viable AD cells with high intensity and reacted with the occasional 9S cell (less than 10%) (results not shown), suggesting that since the antibody was reactive with non-permeabilized cells, it detected a cell surface epitope. To quantitate the degree of reactivity and to confirm the cell surface location of the reactive epitope, flow cytometry was performed (Figure 1). MAB 7.4.1 labelled 84% of the viable AD cells, and only 1–2% of the 9S cells. The mean fluorescence intensity observed with 9S cells was similar to control values and was markedly less than that observed with AD cells.

Since human tumour samples are frequently preserved by fixation, it was of interest to determine whether formalin, glutaraldehyde or methanol affected the epitope recognised by MAB 7.4.1 (Table 1). The antibody had reduced reactivity in a cell ELISA on formalin fixed cells compared to unfixed cells. Moreover, reactivity on cells fixed for 60 min was

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Figure 1 Flow cytometry following indirect immunofluorescence labelling of 9S (-----) and AD (——) cells with NS-1 myeloma culture supernatant supplemented with irrelevant murine Igs (left panel) or MAB 7.4.1 (right panel). Undiluted hybridoma culture supernatant was added to 1×10⁶ 9S and AD cells at 4°C and incubated for 60 min. The cells were washed and FITC-conjugated goat anti-mouse Ig (G + A + M) diluted 1:25 was added. After 60 min, the cells were washed and analysed using a Becton-Dickinson fluorescence activated cell sorter (FACS) IV. The excitation wavelength was 488 nm and the fluorescence detector used a 530/20 nm bandpass filter. The instrument was calibrated before each analysis using a standard suspension of glutaraldehyde-fixed chicken erythrocytes. The frequency distribution of the relative fluorescence of a minimum of 10,000 cells was obtained.
diminished compared to cells fixed for only 15 min. MAb 7.4.1 retained approximately 60% of its reactivity on glutaraldehyde fixed cells compared to control values obtained with unfixed cells. The effect of methanol on MAb 7.4.1 reactivity varied from experiment to experiment, although in most cases, reactivity was diminished 70–100%. Thus, all three fixatives were found to adversely affect antibody reactivity suggesting a limited potential of MAb 7.4.1 as an immunodiagnostic tool in vitro.

Immunoblotting experiments showed that MAb 7.4.1 reacts with three proteins of estimated molecular weights of 186, 169 and 158 kDa (Figure 2). No differences were observed between samples tested under reducing and non-reducing conditions. It is possible that the two smaller proteins represent proteolytic breakdown products of the 186 kDa protein. However, this seems unlikely since protease inhibitors were included in the lysis buffer used to prepare cell extracts. A second possibility is that the difference bands may reflect different degrees of glycosylation of a single protein. Finally, it is also possible that MAB 7.4.1 recognises a common epitope on three unrelated proteins. Further investigation is required to determine which of these explanations accounts for the multiplicity of bands observed. An immunoblot performed under identical conditions but incubated with MAB C219 against P-glycoprotein showed a single broad band of estimated molecular weight 168 kDa on membrane preparations from AD cells (Figure 2).

The ability of MAB 7.4.1 to cross-react with other cell lines was examined by indirect cell ELISA (Figure 3). The cell lines tested included human cell lines H69, HT1080, WiDr and 8226/S as well as the Chinese hamster ovary (CHO) cell line Aux B1 and their respective drug-resistant variants, i.e. H69AR (Mirska et al., 1987), HT1080/DR4 (Slovak et al., 1988), WiDr/R (Dalton et al., 1986), 8226/R40 (Dalton et al., 1986) and CHC5 (Ling & Thompson, 1974). MAB 7.4.1 showed strong reactivity with both the drug-sensitive and -resistant fibrosarcoma cell lines, HT1080 and HT1080/DR4. Both colon carcinoma cell lines were also positive although reactivity was considerably greater on the drug-resistant WiDr/R than its sensitive parent. No reactivity was observed with the myeloma cell lines, 8226/S and 8226/R40, the CHO cell lines, Aux B1 and CHC5, or the small cell lung tumour cell lines, H69 and H69AR. Lastly, MAB 7.4.1 did not cross-react with peripheral blood lymphocytes (results not shown).

In summary, three lines of evidence indicate that MAB 7.4.1 recognises an antigen distinct from P-glycoprotein. Firstly, its pattern of reactivity on immunoblots is quite distinct from that of MAB C219 which is directed towards a highly conserved epitope of P-glycoprotein. Secondly, MAB...
P-glycoprotein is not detectable (Dalton et al., 1988; Slovak et al., 1988). WiDr/R is a mitoxantrone-selected drug-resistant colon carcinoma cell line which does not exhibit the MDR phenotype, as it is commonly defined (Gerlach et al., 1986b), since it displays only marginal cross-resistance to the Vinca alkaloids (Dalton et al., 1988). Nevertheless, the antigen(s) defined by MAb 7.4.1 is overexpressed on this resistant cell line compared to its parent cell line. By contrast, MAb 7.4.1 is only slightly more reactive with drug-resistant fibrosarcoma cell line, HT1080/DR4, compared to its parent cell line, HT1080. One interpretation of these results is that the MAb 7.4.1-defined antigen(s) may be only one of several factors mediating drug resistance in these cell lines. Whether this antibody detects the same set of proteins on the HT1080 and WiDr/R cell lines is currently under investigation. It should be noted that this antigen is not invariably associated with non-P-glycoprotein-mediated drug resistance since it is not found on the MDR small cell lung cancer cell line, H69AR (Mirska et al., 1987). Clearly much work remains to be done to determine what role, if any, the antigen(s) defined by MAb 7.4.1 plays in drug resistance.

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