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Detection of Drug Resistance in Human Ovarian Carcinoma

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Summary. Drug-resistant cancer cells with the multidrug-resistance phenotype show overexpression of *P*-glycoprotein, and we therefore tested carcinoma tissue from five patients with stage III or IV ovarian cancer for *P*-glycoprotein using 265/F4 and C 219 monoclonal antibodies, prepared against membrane glycoproteins in colchicine-resistant CHO cells. Using immunofluorescence and immunoblotting techniques, one of the tumors showed a positive reaction. Using the pcDR 1.5 clone we found that the same cancer tissue had elevated expression of the genes responsible for multidrug resistance. The demonstration of elevated *P*-glycoprotein in ovarian carcinomas indicates that *P*-glycoprotein overexpression is not limited to experimental tumor models.

Key words: Human ovarian carcinoma – Multidrug resistance – *P*-glycoprotein – Monoclonal antibodies

Introduction

Although the statistical probability of successful treatment of ovarian carcinoma is known, the clinical response of the individual patient is uncertain. That is why many workers have tried to develop systems to test the resistance of tumors to cytostatic agents [10, 18, 22, 15]. But no single test system has gained widespread clinical acceptance.

In the last few years the concept of multidrug resistance has emerged and different methods have been developed to detect multidrug resistance of tumors [3, 17]. The most frequently reported characteristic of multidrug resistant cells is the overexpression of a 170 kDa glycoprotein (*P*-glycoprotein) originally described by Ling and coworkers [11]. While drug-resistance has been extensively studied in animal and human tissue cultures, information about the behaviour of

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actual human tumor tissue is sparse [2, 7, 14, 24]. Bell and coworkers [2] using Western-blotting found high levels of *P*-glycoprotein in two out of five human ovarian carcinomas resistant to chemotherapy. In one of the tumors the amount of *P*-glycoprotein increased after further exposure to cytostatic agents.

Using two different monoclonal antibodies (265/F4 and C 219, respectively) and the streptavidin-biotin-phycoerythrin-fluorescence method and immunoblotting (Western-blots) we examined *P*-glycoprotein levels in the tumor tissue obtained from five patients with ovarian carcinoma not previously treated by chemotherapy. We also measured the expression of multidrug-resistant genes using the pcDR 1.5 clone by Northern-blots.

Material and Methods

Surgical Specimens. Tumor tissue was obtained (by Dr. W. Kleine) from five patients with stage III or IV ovarian carcinoma operated on in the Department of Obstetrics and Gynecology of the University of Freiburg. All tumor tissue was stored at −80°C.

Immunofluorescence. For the immunofluorescent detection of *P*-glycoprotein we used the streptavidin-biotin-phycoerythrin method recently developed by Amersham (Braunschweig, FRG). After airdrying, the cryostat sections were fixed in acetone, then incubated with normal sheep serum and finally with the primary monoclonal antibodies (265/F4 or C 219) (10 μg/ml, 2 h). After washing, the cells were incubated with biotinylated sheep antimouse second antibody (dilution 1:50, 30 min, Amersham, pooled with 5% human IgG) and after rewashing in PBS, the streptavidin-biotinylated phycoerythrin-complex (Amersham) was added (dilution 1:50, 40 min). After treatment with a stabilizer for 20 min to prevent rapid fading of phycoerythrin-fluorescence, the slides were dried and mounted. The preparation and characterization of the monoclonal antibodies have been described earlier [12, 13]. The antibodies were kindly donated by Dr. B. Lathan, Cologne, FRG and Dr. V. Ling, Toronto, Canada. These antibodies were prepared against the membrane glycoproteins in colchicine-resistant Chinese hamster ovary (CHO) cells.

Western-blots. This method has been described earlier in detail [25]. The isolation of plasma membranes was performed according to a slight modification of the method of Riordan and Ling [16]. SDS-PAGE was carried out in a slab gel apparatus according to Fairbanks [6]. Protein concentration in the different protein extracts was determined according to the method of Bradford [4] and immunoblotting was performed according to the methods of Towbin et al. [21] and Lathan et al. [13].

Northern-blots. RNA was isolated from tissue by the guanidinium isothiocyanate method. For Northern blot analysis RNA was separated by electrophoresis on formaldehyde gels and transferred to nitrocellulose membranes which were subsequently hybridized with the ^{32}P-labeled pcDR 1.5 (the probe was provided by Dr. Croop, Cambridge, Mass., USA). The isolation of the probe has been described earlier [8, 9]. The hybridization was carried out as described in an earlier report [23].

Results

Expression of P-glycoprotein

Of the five untreated ovarian carcinomas, one showed an intense immunofluorescent reaction (Fig. 1). Identical results were obtained by both 265/F4 and

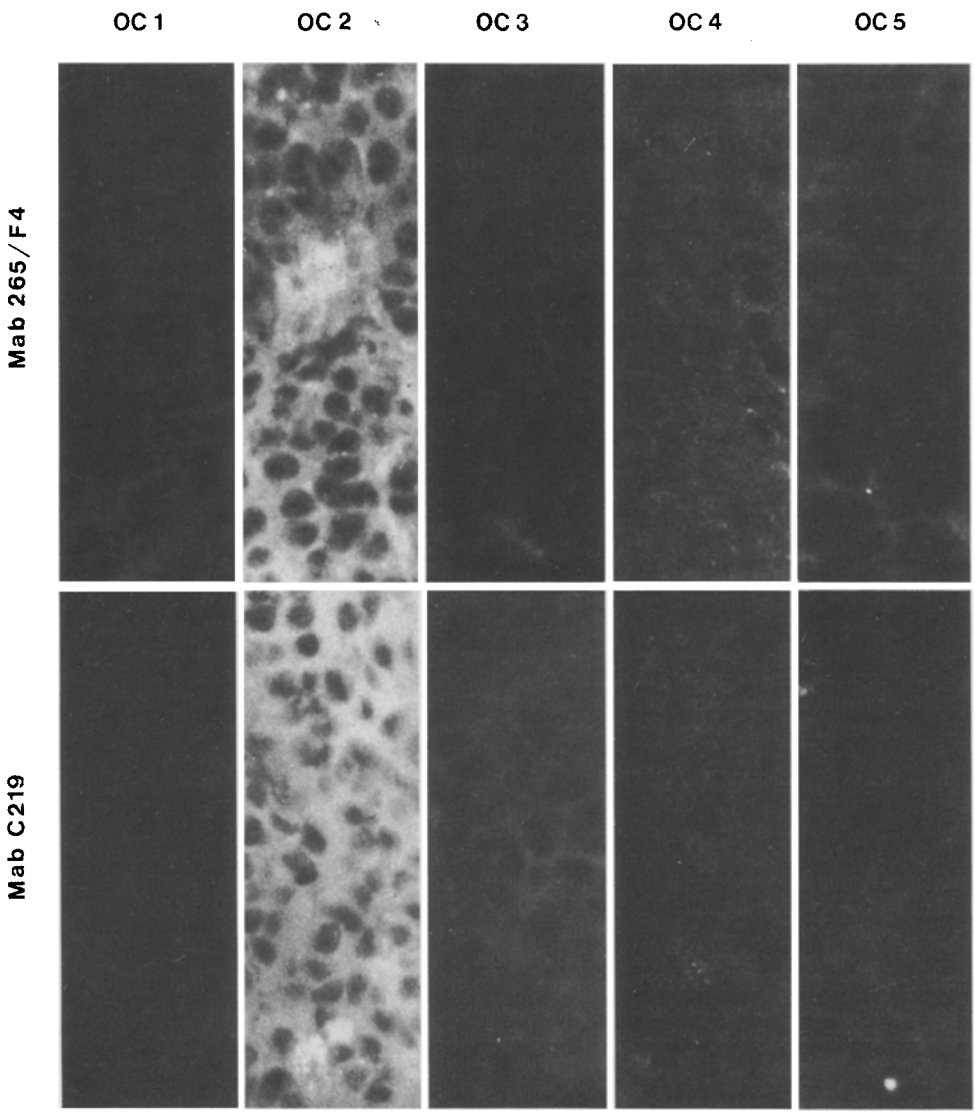

Fig. 1. Immunofluorescence with Mab 265/F4 (top) and with Mab C 219 (bottom) of five human ovarian carcinomas (OC) before chemotherapy. Magnification : 500 X. Only tumor 2 shows a positive reaction

C 219 monoclonal antibodies. These results were confirmed by Westernblotting with Mab 265/F4 (Fig. 2, top) and Mab C 219 (data not shown).

Expression of Multidrug-resistant DNA-sequences

To determine whether multidrug-resistant DNA-sequences were expressed in untreated ovarian carcinomas, Northern-blots were performed using the pcDR

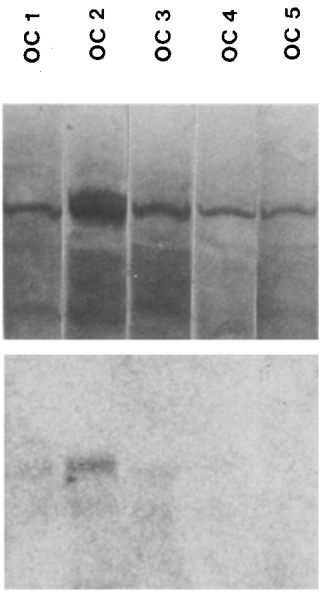

Fig. 2. Western-blots (top) with Mab 265/F4 and Northern-blots (bottom) hybridized with the labeled pcDR 1.5 probe (conc. 10 μg protein and 10 μg RNA pro lane). Tumor 2 shows a positive reaction

1.5 clone (Fig. 2, bottom). Expression of the genes responsible for multidrug resistance was detected in the same ovarian carcinoma which showed an increase of *P*-glycoprotein. Lack of difference in β-actin gene expression suggests that comparable levels of RNA were loaded onto the gels.

Discussion

Tumor cells can acquire resistance to a wide range of compounds which have no obvious structural or functional similarities (e.g. alkaloids, anthracyclines and antibiotics). This phenomenon seems to be associated with a decreased net cellular concentration of the drugs. Danø [5] and Skovsgaard [20] could demonstrate, by using metabolic inhibitors, that this drug efflux represents an energy-dependent carrier-mediated transport mechanism. The modified transport mechanism exhibited in resistant cells is caused by changes in the composition of plasma membrane [1, 19, 26]. Several reports have indicated a correlation between the existence of *P*-glycoprotein and a multidrug-resistance phenotype [17, 23]. Bell et al. [2] using Western blot analyses detected *P*-glycoprotein in two out of five patients with treated solid ovarian carcinomas. In the present study we analyzed the distribution of *P*-glycoprotein in ovarian carcinoma not previously treated with cytostatic agents. By means of immunofluorescence and immunoblotting using 265/F4 and C 219 antibodies we found that one out of five ovarian carcinomas had a higher expression of *P*-glycoprotein. Using the pcDR 1.5 cDNA probe we also measured the expression of DNA sequences associated with multidrug-resistance and found that multidrug DNA sequences were expressed only in this ovarian carcinoma. Thus, the *P*-glycoprotein can also be

detected in ovarian carcinomas not previously treated with chemotherapy. After surgery all five of our patients were treated with cytostatic agents which were not involved in the multidrug resistance pattern and it is not possible to relate drug resistance and the expression of *P*-glycoprotein in this study.

By measuring the *P*-glycoprotein one could, in theory, have a method identifying tumors which might fail to respond to chemotherapy, but we have no evidence in this study to support such a claim. Such measurements would have the advantage that they could be done directly on tissue biopsies and would therefore not require cell culture techniques. Further studies are required to optimize the detection of *P*-glycoprotein in ovarian cancer samples and to establish the relationship between level of *P*-glycoprotein expression and clinical outcome to chemotherapy. Nevertheless, the demonstration of elevated *P*-glycoprotein in non-treated and treated ovarian carcinomas indicates that *P*-glycoprotein overexpression in not limited to experimental tumor models.

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