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The expression of metallothionein (MT) and proliferation intensity in ovarian cancers treated with cisplatin and paclitaxel

Paweł Surowiak[1], Irina Kaplenko[2], Marek Spaczyński[2], Maciej Zabel[1, 3]

[1]Chair and Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland
[2]Chair and Department of Gynaecology and Obstetrics, University of Medical Sciences, Poznań, Poland
[3]Chair and Department of Histology and Embryology, University of Medical Sciences, Poznań, Poland

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Metallothioneins (MT) represent low molecular weight proteins that are supposed to fulfil several functions. They participate in the cell cycle, protect cells from oxidative stress, control levels of heavy metals and participate in multidrug resistance processes, particularly in cases of alkylating drugs. The present study aimed at evaluation of proliferation intensity (Ki67, PCNA) in ovarian cancers treated using cisplatin and paclitaxel, as related to expression of MT.

The experiments were performed on samples originating from 10 patients operated on due to ovarian cancer. The material originated from the first operations or second-look operations. All the patients were treated with cisplatin and paclitaxel. Immunocytochemical reactions using antibodies to MT, Ki67 and PCNA were performed in paraffin sections originating from the cases studied. Statistical analysis was performed using Statistica software. The studies demonstrated no relation between expression of MT on the one hand and intensity of proliferation before or after chemotherapy on the other hand (gamma correlation, $p > 0.05$). The results indicate that expression of MT is not related to resistance to treatment using cisplatin and paclitaxel.

key words: ovarian cancer, metallothionein, proliferation, cisplatin, paclitaxel

INTRODUCTION

Ovarian cancer occupies 3rd place in the list of morbidity due to malignant tumours of the genital system in women in Western and Northern Europe and in USA and 5th place among all malignant tumours in women. Worldwide, the tumour exhibits persisting growth in incidence. In Poland in 1992 the incidence was 11.1 per 100,000 women [5]. The location of ovarian cancers leads to the tumours being recognised only at an advanced stage of their development. Therefore, in a significant fraction of cases of the disease surgery has to be accompanied by chemotherapy. At present the most popular drug set used in the treatment of ovarian cancer includes paclitaxel and cis- or carboplatin [4].

Numerous studies have suggested a relation between increased expression of metallothioneins (MT) and resistance of tumour cells to cytostatic drugs and, in particular, alkylating drugs (cis- and carboplatin) [2]. Metallothioneins are low molecular weight proteins (6–7 kDa) of a chain of 61 or 62 amino acids. They are characterised by a high content of cystein residues and a low content of aromatic amino acids. Their unique property involves capacity to bind heavy metals [3]. Hagrman et al. [1] noted that MT may also bind cisplatin molecules. This mechanism might ex-

Address for correspondence: Paweł Surowiak, MD, Chair and Department of Histology and Embryology, University School of Medicine, ul. Chałubińskiego 6a, 50–356 Wrocław, Poland, tel: +48 71 784 00 81, fax: +48 71 784 00 82, e-mail: pawel.surowiak@interia.pl

plain the potential linkage between expression of MT and the resistance of tumours to alkylating drugs.

The present study aimed at evaluating the intensity of proliferation markers (Ki67, PCNA) in samples of ovarian cancers originating from first operations or from second-look operations following treatment with cisplatin and paclitaxel, as related to the expression of MT.

MATERIAL AND METHODS

The studies were performed on samples of ovarian cancers originating from 10 patients subjected to surgery in 2000–2001, in the Department of Gynaecology and Obstetrics, University of Medical Sciences, Poznań (Table 1). In all the cases H+E--stained preparations were made, which were examined by two pathologists. In paraffin sections originating from the studied cases and obtained during the first operations and upon second-look operations, immunocytochemical reactions were performed using mouse monoclonal antibodies against MT (clone E9), Ki67 (clone MIB-1) and PCNA (clone PC10). In every case a negative control was run, using the Mouse Primary Negative Control. The antigens under investigation were visualised employing LSAB2 and DAB kits. In the cases of Ki67 and PCNA the sections being studied were boiled in Antigen Retrieval Solution. All the reagents originated from Dako Cytomation, Denmark. MT expression was quantitated using the semiquantitative IRS scale (Table 2) [6], while in the cases of Ki67 and PCNA the percentage of positive cells was taken into account. The results obtained were subjected to statistical analysis using the Statistica 98 PL software.

Table 1. Studied cases — diagnoses

First operation	Second-look operation
Carcinoma solidum adenoides	Adenocarcinoma serosum partim solidum
Adenocarcinoma endometroides	Cystadenocarcinoma endometrioidale
Adenocarcinoma serosum	Adenocarcinoma papillare serosum
Adenocarcinoma serosum	Adenocarcinoma serosum
Adenocarcinoma serosum	Foci carcinomatosi
Carcinoma solidum adenoides serosum	Carcinoma solidum adenoides clarocellulare
Adenocarcinoma serosum	Adenocarcinoma endometroides
Adenocarcinoma serosum	Adenocarcinoma clarocellulare
Adenocarcinoma serosum	Adenocarcinoma serosum
Adenocarcinoma serosum	Adenocarcinoma serosum

RESULTS AND DISCUSSION

The immunocytochemical reactions performed enabled the following pattern of reactions to be documented:

— in the case of MT colour reaction was obtained in the cell nuclei and in the cytoplasm, of variable intensity in individual cases (Fig. 1, Table 3);
— in the cases of Ki67 and PCNA colour reaction was obtained localised in the cell nuclei, of variable intensity in individual cases (Fig. 2, 3, Table 3).

Table 2. Quantitation of immunocytochemical reaction intensity using ImmunoReactiv Score scale according to Remmele

Percentage of positive cells	Score	Reaction intensity	Score
No positive cells	0	No reaction	0
< 10% positive cells	1	Weak colour reaction	1
10–50% positive cells	2	Moderate intensity of colour reaction	2
51–80% positive cells	3	Intense colour reaction	3
> 80% positive cells	4		

Table 3. Mean intensity of immunocytochemical reactions in the studied groups

Tested antigen	First operation	Second-look operation
MT	5.9 ± 3.98	5.2 ± 3.36
Ki67	42.3 ± 29.94	11.1 ± 10.64
PCNA	61 ± 30.26	54 ± 28.75

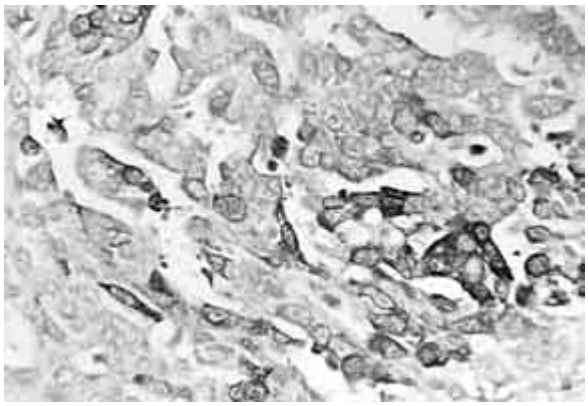

Figure 1. Immunocytochemical localisation of metallothionein in cells of ovarian cancer (× 400).

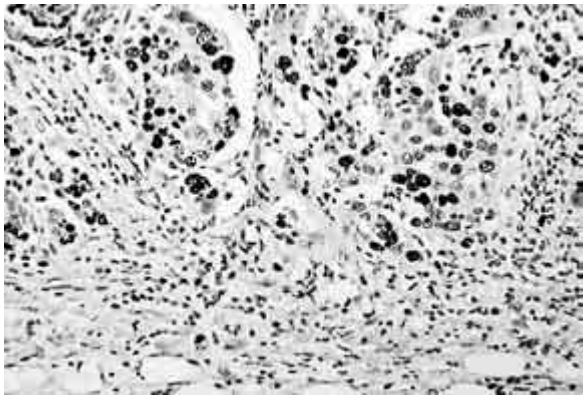

Figure 2. Immunocytochemical localisation of Ki67 in cells of ovarian cancer (× 200).

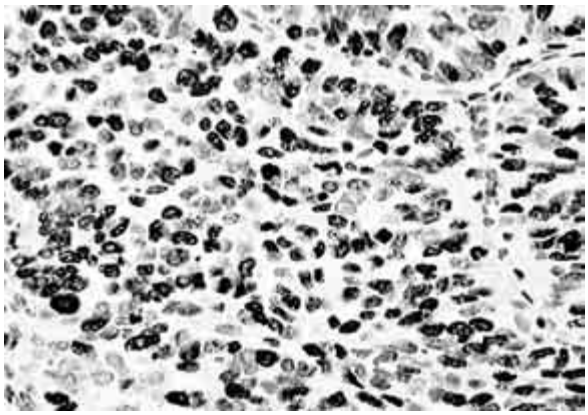

Figure 3. Immunocytochemical localisation of PCNA in cells of ovarian cancer (× 200).

Statistical calculations failed to document a relationship between MT expression and intensity of proliferation in individual groups and between the groups (gamma correlation; $p > 0.05$). Using the Mann-Whitney U test, it was demonstrated that only in the case of Ki67 did intensity of expression differ between the groups, as it was significantly lower in material obtained in second-look operations (following chemotherapy; $p = 0.02$).

The references quoted in the Introduction described a relationship between expression of MT in tumour cells and resistance to alkylating drugs [1, 2]. In our study proliferative antigens (Ki67 and PCNA) served as an index of sensitivity to chemotherapy. No relationship could be found between expression of MT and that of Ki67 and PCNA. The lack of any relationship suggests that a weak relation or no relation links MT expression and resistance to cisplatin. It is probable that proteins from the group of ABC transporters play a more significant role in this phenomenon.

In our study we have shown that expression of Ki67 has been significantly lower following chemotherapy. No such differences could have been observed in PCNA. Most probably Ki67 represents a more reliable index of proliferation.

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