the protocols; therefore, the analysis of the results did not show any beneficial role of nitrosourea [9].

In conclusion, the present results and those in the literature confirm that cystemustine should not be used in the treatment of colorectal carcinomas.


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# The Immunohistochemical Expression of Proliferating Cell Nuclear Antigen (PCNA/Cyclin) in Malignant and Benign Epithelial Ovarian Neoplasms and Correlation with Prognosis

**L. Nakopoulou, J. Janinis, G. Panagos, G. Comin and P. Davaris**

**Proliferating cell nuclear antigen (PCNA)/cyclin is considered to be a marker of cell proliferation. The aim of this study was to evaluate the expression of PCNA/cyclin in epithelial ovarian neoplasms (EON) as well as the possible correlation with degree of differentiation, tumour stage and overall survival. The material consisted of 34 benign and 40 malignant EON. Positive nuclear staining was detected in 2/34 (6%) of benign and 23/39 (59%) malignant EON ($P < 0.001$). Most cases in the high proliferation group were diagnosed in advanced clinical stages. There was no difference in overall survival between nuclear PCNA positive and negative patients, as well as the high and the low proliferation group. In conclusion, the role of PCNA as a marker of malignant potential and prognosis in EON merits further investigation.**
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## INTRODUCTION

CURRENTLY THERE is growing information regarding monoclonal antibodies that recognise a 36 kD, S-phase associated, nuclear protein called proliferating cell nuclear antigen (PCNA)/cyclin. PCNA/cyclin plays a critical role in DNA synthesis and the initiation of cell proliferation [1]. PCNA/cyclin has been detected immunohistochemically on paraffin sections of normal and malignant tissues such as gastrointestinal lymphomas, breast, gastric, pancreatic, prostatic and renal cell carcinomas and haemangiopericytomas [2–7].

The association of PCNA/cyclin with the rate of proliferation using monoclonal anti-PCNA/cyclin antibodies has been investigated with the aid of immunofluorescence microscopy and flow cytometry [8, 9]. Monoclonal antibodies PC-10 and 19A2 that recognise the acidic protein of PCNA have shown a linear correlation with other proliferation indices such as Ki-67 and flow cytometry in lymphoid malignancies, whereas in other tumours such as breast and gastric carcinomas no such correlation has been found [2].

The aim of this study was to investigate PCNA expression in benign and malignant epithelial ovarian neoplasms (EON). In addition, an attempt was made to study the possible correlation of PCNA with other parameters such as histological type and grade, clinical stage and overall survival.

## MATERIALS AND METHODS

A retrospective analysis of tumour tissue obtained from the Department of Pathology, University of Athens Medical School was performed. The tissue specimens were obtained from a total of 73 women with EON of which 34 were benign and 39

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malignant. All patients with malignant EON were clinically staged according to FIGO classification. After deparaffinisation, sections were subsequently washed in phosphate buffered saline (PBS). Immunostaining was performed using the ABC-HRP method (Dakopatts, Denmark). The monoclonal antibody PC-10 (Dako-PCNA, PC-10) was used as primary antibody at a dilution of 1:150 with a 24 h (over-night) incubation.

All immunostained slides were analysed and scored in a blinded fashion without knowledge of histological type and grade or survival data. Analysis of the immunostained sections were based on the number of stained nuclei. In each section 1000 cells were counted and the fraction of positive cells was determined. Two sections per case were evaluated and scored and the average number from both sections was used. Cases with less than 5% positive nuclei were regarded as negative. According to the percentage of positively stained nuclei, malignant EON were classified into a low proliferation group (less than 25% positive nuclei) and a high proliferation group (> 25% positive nuclei). Statistical analysis was performed using the $\chi^2$ test.

## RESULTS

The expression of PCNA in the neoplastic cells was heterogeneous with most of the positive stains being nuclear while cytoplasmic staining was less frequent. Positive nuclear staining for PCNA was detected in 6% of benign and 59% of malignant EON (Table 1). This difference in nuclear PCNA expression between benign and malignant EON was statistically significant ($P < 0.001$). Nuclear PCNA expression was more pronounced in malignant serous, endometrioid and undifferentiated EON, while it was absent in all malignant mucinous and benign Brenner tumours. Nuclear staining demonstrated either a diffuse or a granular pattern or a combination of both. Heterogeneity within the same tumour specimen was also noted. Cytoplasmic PCNA staining was detected in 26% of benign, and 38% of malignant EON (Fig. 1). Positive PCNA expression was found in 3/6 (50%) stage I, 4/5 (80%) stage II, 12/20 (60%) stage III and 1/1 stage IV patients. The majority of cases in the high proliferation group were in advanced clinical stages. The median survival of patients with malignant EON and positive PCNA nuclear expression was 18 months versus 19 months for those with negative PCNA expression, and 15 months for those with positive cytoplasmic PCNA expression versus 20 months for those with negative expression. There was no difference in survival between the low and the high proliferation group.

*Table 1. Distribution of PCNA in benign and malignant EON*. Correlation with histological type degree of differentiation and tumour stage*

| Histological type | No of patients | Mean age | PCNA expression Nuclear | PCNA expression Cytoplasmic | Proliferation Group Low | Proliferation Group High |
|---|---|---|---|---|---|---|
| Benign | | | | | | |
| Serous | 19 | 54 | 1 | 7 | | |
| Mucinous | 10 | 55 | 1 | 0 | | |
| Brenner | 5 | 69 | 0 | 2 | | |
| Total benign | 34 | 70 | 2 | 9 | | |
| Malignant | | | | | | |
| Serous | 20 | 56 | 12 | 7 | 0 | 12 |
| Well diff. | 8 | | | | 5 | 3 |
| Moderately diff. | 8 | | | | 3 | 5 |
| Poorly diff. | 4 | | | | 1 | 3 |
| Mucinous | 3 | 72 | 0 | 12 | 0 | 0 |
| Endometrioid | 8 | 62 | 6 | 2 | 1 | 5 |
| Mixed | 3 | 64 | 1 | 1 | 1 | 0 |
| Clear cell | 2 | 57 | 1 | 2 | 1 | 0 |
| Undifferentiated | 3 | 55 | 3 | 1 | 1 | 2 |
| Total malignant | 39 | 61 | 23 | 15 | 4 | 19 |
| Clinical Stage FIGO | | | | | | |
| I and II | 11 | | 7 | | 3 | 4 |
| III and IV | 21 | | 13 | | 2 | 11 |

* Epithelial ovarian neoplasms.

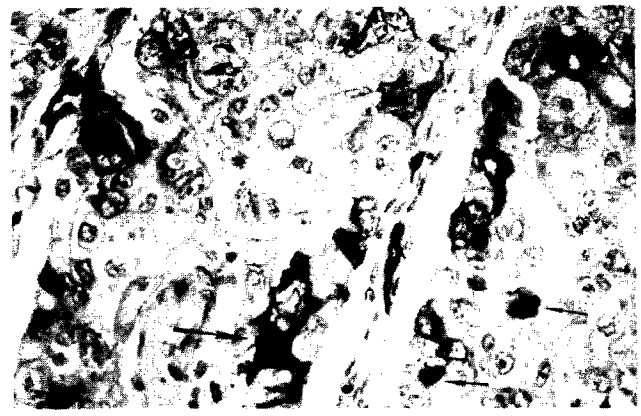

**Fig. 1. Undifferentiated carcinoma: positive cytoplasmic and membranous PCNA expression (large arrows) and rare nuclear expression (small arrows) (ABC × 500).**

## DISCUSSION

A gradient in PCNA expression was found among neoplastic cells within the same tumour specimen (intrinsic heterogeneity). This observation indicates that there are subpopulations of cells at different phases of cell proliferation. Similar findings have been reported in other studies [8] indicating varying levels of PCNA/cyclin during the cell cycle, being lowest during mitosis and highest during the S-phase.

The presence of PCNA expression in some benign tumours can be partially explained by the long half life of the PCNA protein of 20 h [10]. As a result of this PCNA can be immunologically detected in cells after they have left the cell cycle.

A positive correlation was found between PCNA expression and histological grade in malignant serous EON. This is of particular importance since other investigators have shown that histological grade correlates adversely with 5 year survival in malignant serous EON [11].

The significance of cytoplasmic PCNA staining is uncertain. Hall *et al.*, and Chan *et al.* separately reported a cytoplasmic staining pattern, the nature of which is unclear, though it could represent cytoplasmic synthesis or breakdown [2, 6]. It could also be attributed to mitotic cells in which the nuclear membrane is lost during mitosis with resulting diffuse staining throughout the cells. Recent studies have shown that cyclin B accumulates in the cell cytoplasm and enters the nucleus during M-phase [12]. Finally, local ovarian specific mechanisms are also likely to be involved.

Of interest is Zuber *et al.*'s observation that in the meiosis arrested ova PCNA is stockpiled for use in early embryogenesis [13]. In our study cytoplasmic staining was found in a considerable number of cases. The lack of a statistically significant difference in cytoplasmic PCNA staining between benign and malignant EON undermines its importance as a discriminating marker for malignant EON.

A positive correlation was found between advanced clinical stages and increased PCNA expression. This is explained by the biological aggressiveness and invasive potential of tumours in the high proliferation group.

Since the prognostic value of PCNA expression is still inconclusive, we suggest that prospectively collected data with larger series of patients should be accumulated before assigning PCNA an important role as a biological prognostic factor in ovarian cancer.


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# Hypercalcaemia in Small Cell Lung Cancer: Report of a Case Associated with Parathyroid Hormone-related Protein (PTHrP)

**R. Stuart-Harris, V. Ahern, J.A. Danks, H. Gurney and T.J. Martin**

**Although hypercalcaemia is frequently associated with malignancy, it is very rare in small cell lung cancer despite the high incidence of lytic bone metastases. We report a patient with extensive small cell cancer who presented with hypercalcaemia. Investigations suggested parathyroid hormone (PTH) receptor stimulation, although the serum PTH level was not elevated. PTH related protein (PTHrP) was localised in a biopsy specimen from the tumour. Although hypercalcaemia is rare in small cell lung cancer, when hypercalcaemia does occur, PTHrP may be a causal factor.**

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## INTRODUCTION

HYPERCALCAEMIA IS a frequent complication of many solid tumours, especially renal cancer, breast cancer and non-small cell lung cancer. Overall, approximately 12% of patients [1] with primary lung cancer develop tumour-induced hypercalcaemia (TIH). TIH is particularly common in squamous cell carcinoma of the lung, where up to 25% of patients develop hypercalcaemia at some time during the course of their illness. TIH is principally induced by increased bone resorption or reduced renal calcium excretion, but more than one mechanism may occur in any one patient. Although bone metastases are frequently present, it is now known that hormonal factors are important in the majority of cases of TIH, whether or not bone metastases are present. Parathyroid hormone (PTH) related protein (PTHrP) has recently been implicated as one of the main humoral factors causing TIH [2].

Small cell lung cancer (SCLC) is a common malignancy with a propensity to early dissemination especially to bone, and is also commonly associated with ectopic hormone production. By the use of specific immunohistochemical staining, some SCLC biopsy specimens have been shown to contain PTHrP [3]. However, hypercalcaemia is a rare complication.