

## EXPRESSION OF CYCLIN D1 AND KI-67 IN ENDOMETRIAL HYPERPLASIAS AND CARCINOMAS

RALUCA BALAN<sup>1</sup>, CORNELIA AMALINEI<sup>1</sup>, EDUARD CRAUCIUC<sup>1</sup>,  
IRINA DRAGA CARUNTU<sup>1</sup>, VLAD GHEORGHITA<sup>2</sup>, OVIDIU TOMA<sup>3</sup>

**Keywords:** endometrial carcinoma, endometrioid carcinoma, serous papillary carcinoma, hyperplasia, immunohistochemistry, cyclin D1, Ki-67

**Abstract:** Endometrioid and serous carcinomas of the endometrium differ dramatically in their pathogeny and clinical behaviour. CyclinD1 is critical for the G1/S part of the cell cycle. Cyclin D1 is often overexpressed in human neoplasias, by genes rearrangements, amplifications and mutations. Ki-67 is a cell proliferation marker that is positive in all phases of the cell cycle except G<sub>0</sub>. The purpose of this study was to compare endometrial carcinomas with endometrial hyperplasias, regarding their activity for cyclin D1 and Ki-67. Immunostains with Ki-67 and cyclin D1 antibodies were performed on formalin-fixed and paraffin-embedded tissue sections from 15 cases: 10 endometrial carcinomas, endometrioid and serous papillary, and 5 simple and complex hyperplasias, with and without cytologic atypia. Ki-67 was positive in all proliferative and neoplastic endometria. It is emphasized that the association of the cyclin D1 expression with the clinical aggressiveness is possible, but insufficient proved by now. Overexpression of cyclin D1 increases from normal endometrium to hyperplasia and carcinoma, suggesting that it may play a role in endometrial carcinogenesis.

### INTRODUCTION

Endometrial carcinoma (EC) includes at least two distinct subtypes, each of these being associated with different risk factors, precursor lesions, morphology, evolution, and molecular genetic attributes. Type I, the endometrioid carcinoma, which represents 80% from endometrial carcinomas, is often diagnosed in stage I, usually is associated with hyperestrogenism and appears at younger women, in premenopause. Unlike this, type II, represented by serous papillary endometrial carcinoma, which is often very aggressive and appears in older women, in postmenopause, is developed on an atrophic milieu and is considered not to be significantly dependent on estrogens for proliferation (1). Many researchers suggest that type I of endometrial tumors are developed from endometrial hyperplasias in a context of hyperestrogenism, having usually an indolent clinic evolution (2). On the other hand, serous endometrial carcinomas are developed on an atrophic background, from the morphologic entity named „endometrial intraepithelial carcinoma”, which represents the malignant transformation of surface endometrial epithelium (3). Numerous studies indicated that the differences in immunohistochemical profiles of endometrial carcinomas, endometrioid and serous, support the idea of existence of different molecular pathways of development (4).

### MATERIAL AND METHODS

Our study comprises 15 cases (10 cases of endometrial carcinomas – 5 endometrioid carcinomas well differentiated (WDEC), three endometrioid carcinomas moderate differentiated (MDEC), two serous papillary carcinomas (SPEC), 5 cases of hyperplasias – one simple hyperplasia (SHP) without atypia, one simple hyperplasia with atypia, one complex hyperplasia (CHP) without atypia, two complex hyperplasias with atypia). The patients were diagnosed, investigated through endometrial biopsy and surgically treated at The 3<sup>rd</sup> Clinic of Obstetrics and Gynecology Iasi. The specimens were routinely fixed in 20% buffered formalin solution and paraffin embedded and stained with hematoxylin and eosin. Immunostains with Ki-67 and cyclin D1 antibodies were performed on routinely processed tissue sections for all presented cases. It was also performed a semiquantitative evaluation; the statistical interpretation of the data was done through student t test, unpaired. In accordance with immunohistochemical working protocols, there were evaluated the expressions of Ki-67 (DAKO, clone Ki-S5, dilution 1:25) and cyclin D1 (DAKO, clone DCS-6, dilution 1:25) in each of the endometrial specimens. The sections were dewaxed, rehydrated in gradual alcohol solutions and then blocked for endogene peroxidase in 3% hydrogen peroxide. Heat induced antigen retrieval method, using a drying stove, was performed for Ki-67 and cyclin D1. All the tissues were preblocked with diluted normal serum and incubated with primary antibody (Ab) for 60 min at room temperature. Immunoreactive complexes were detected using Envision + (DAKO) and visualised with DAB + (DAKO). The slides for negative control were treated with IgG serum isotype. The positive control resulted from positive immunostaining of red blood cells, being treated in the same manner as the studied cases.

The semiquantitative evaluation of nuclear immunostaining for Ki-67 was done in percentage, estimating as being low (up to 20%), moderate (between 20 and 50%) and strong (over 50%). For cyclin D1, it was considered: + (nuclear immunostaining), ++ (cytoplasmic immunostaining), +++ (nuclear and cytoplasmic immunostaining).

For statistical evaluation of data we used unpaired student t test. This test is usually chosen for comparing two groups in which the individual values are not pairs or they don't correspond some with the other. The unpaired student t

test is one of the most used technique for testing an hypothesis which is based on the difference between medial values of the studied populations.

## RESULTS AND DISCUSSIONS

Cyclin D1 is a promoter of the progression of the G1-S phase and, through this, it has a role in cell proliferation, including the tumoral proliferation. The studied proliferation markers show their presence in all proliferative forms.

If the cancer is considered as a cell cycle disease, the most frequent disturbed phase is the control of the G1/S phase (5). In general cases, the positive effectors of G1/S control, as cyclin D1, are considered the principal protooncogenes, and negative effectors, as CDK inhibitors, are considered tumor suppressors (6, 7).

Soslow (8) analysed the expression of cyclin D1, estrogen receptors (ER) and progesteron receptors (PR) in endometrioid and serous endometrial carcinomas, corresponding in histological grade. The expression of cyclin D1 in endometrial carcinomas is associated with endometrioid histology. Its expression was less studied serous papillary endometrial carcinomas. The activation of cyclin D1 stimulates the transcription of genes regulated by ER and PR. The identification of the differences and the elucidation of the relations between cyclin D1, ER and PR will bring supports for establishment of the molecular profiles of endometrioid and serous papillary endometrial carcinomas. In our study we did not make a comparison between the expression of cyclin D1 and ER and PR. It can not be estimated the effect of cyclin D1 upon the prognostic of moderate to poorly differentiated endometrial carcinomas, but the data suggest that cyclin D1 could be expressed by a subset of endometrioid carcinomas which are not the most aggressive (fact established by the presence of cyclin D1 in endometrioid carcinomas comparative with serous endometrial carcinomas and the absence of cyclin D1 in advanced stages of endometrioid carcinomas) (9). The significance of the expression of cyclin D1 correlated with the biology and clinical aggressiveness was not established.

From this point of view, we have analysed the expression of cyclin D1 and Ki-67 also in serous endometrial carcinomas and different types of endometrial hyperplasias and endometrioid carcinomas. The expression of cyclin D1 was strong in both papillary serous endometrial carcinomas (fig. 6) and endometrial hyperplasias, with cytologic atypia, both simple (fig. 4) and complex. The expression of cyclin D1 was also strong in all grades of endometrioid carcinomas (fig. 2). Regarding the expression of Ki-67, the results support the conclusions that all proliferative states, benign (fig. 5) and malignant (fig. 1,3), express proliferative activity (10).

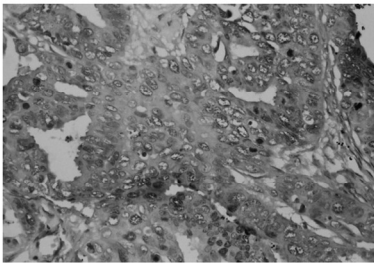


Fig.1 WDEC, Ki-67, strong nuclear immunostaining (X 40)

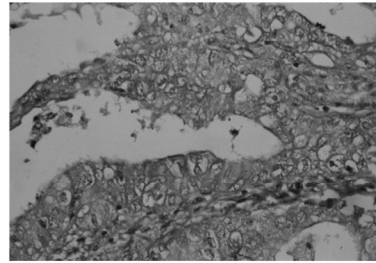


Fig. 2 MDEC, cyclin D1, moderate immunostaining (X40)

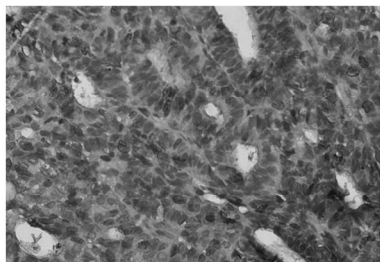


Fig. 3 MDEC, Ki-67, strong nuclear immunostaining (X 40)

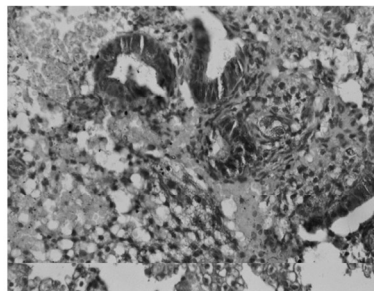


Fig. 4 Atypic SHP, cyclin D1, nuclear and cytoplasmic immunostaining (X 40)

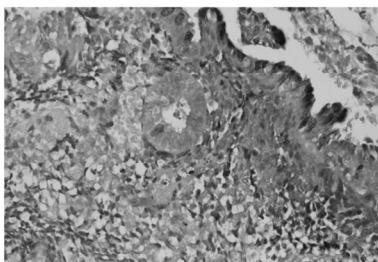


Fig. 5 SHP without atypia, Ki-67, weak immunostaining (X 40)

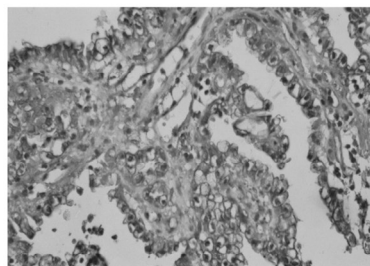


Fig. 6 SPEC, cyclin D1, nuclear and cytoplasmic immunostaining (X 40)

It is considered that the nuclear export of cyclin D1 during S phase of the cell cycle is a critical element in the regulation of normal cellular proliferation. Alt et al (11) demonstrated that the nucleo-cytoplasmic relocalization of cyclin D1 during S phase is mediated by the nuclear export CRM1 (exportin1)-dependent. Cyclin D1 accumulates in the nucleus during G1 phase, but it relocalizes in the cytoplasm in interphase. The specific redistribution of cyclin D1 is correlated with its phosphorylation at Thr-286 by GSK-3 $\beta$  (11).

It is emphasized that the association of the cyclin D1 expression with the clinical aggressiveness is possible, but insufficient proved by now.

Regarding proliferation and cell cycle markers, the results for Ki-67 are exactly as we expected. All proliferative forms, including malignancies (fig. 1,3), present proliferative activity, but without statistic significance between benign proliferative forms and low grade carcinomas (12, 13).

The studied cases permitted the comparison of endometrial carcinomas with endometrial hyperplasias, regarding their activity for cyclin D1 and Ki67. The difference was expressed also by the utilization of the student t test.

The obtained immunohistochemical results for cyclin D1 and Ki-67, are presented in table 1:

**Table 1. The expression of cyclin D1 and Ki-67 in endometrial carcinomas and hyperplasias**

Category	Cyclin D1	Ki-67
WDEC	+++	45%
WDEC	+ and ++	30%

Category	Cyclin D1	Ki-67
WDEC	+	20%
WDEC	Negative	70%
WDEC	+ and ++	15%
MDEC	+++	40%
MDEC	+++	60%
MDEC	+ and ++	20%
SPEC	+++	25%
SPEC	+ and ++	20%
SHP	+	20%
ASHP	+++	25-30%
CHP	+++	55%
ACHP	++	25%
ACHP	++ and +++	55%

Table 2 presents the results of impaired student t test, for the following compared groups for the expression of used markers:

**Table 2. Probability of the t test results, presuming the nule hypothesis**

Markers	Compared groups	p value (statistic significance)
<b>Cyclin D1</b>	WDEC and HP	p = 0,25 (p > 0,05)
	EC and HP	p = 0,58 (p > 0,05)
	MDEC and HP	p = 0,68 (p > 0,05)
<b>Ki-67</b>	MDEC and HPA	p = 0,88 (p > 0,05)
	WDEC and HP	p = 0,94 (p > 0,05)
	EC and HP	p = 0,81 (p > 0,05)
	MDEC and HP	p = 0,72 (p > 0,05)
	MDEC and HPA	p = 0,78 (p > 0,05)

## CONCLUSIONS

Exactly as we expected, it was observed the overexpression of Ki-67 in hyperplastic and neoplastic endometrium.

The expressions of cyclin D1 and Ki-67 studied in serous endometrial carcinomas and different types of endometrial hyperplasias and endometrioid carcinomas were strong, both in serous papillary tumors and in simple and complex hyperplasias, with cytologic atypia. Also, the expression of cyclin D1 was strong in all grades of endometrioid carcinomas, data which sustained the aspects already presented.

It is emphasized that the association of the cyclin D1 expression with the clinical aggressiveness is possible, but insufficient proved by now.

Overexpression of cyclin D1 increases from normal endometrium to hyperplasia and carcinoma, suggesting that it may play a role in endometrial carcinogenesis.

## REFERENCES

- Sherman ME, 2000. Theories of endometrial carcinogenesis: a multidisciplinary approach, *Mod Pathol*, 13(3):295-308
- Kurman J. Robert, 2002. *Blaustein's Pathology of the Female Genital Tract*, 5<sup>th</sup> Edition, Springer-Verlag, 503-535
- Spiegel GW, 1995. Endometrial carcinoma in situ in postmenopausal women, *Am J Surg Pathol*, 19:417-32
- Kounelis S, Kapranos N, Kouri E, Coppola D, Papadaki H, Jones MW, 2000. Immunohistochemical profile of endometrial adenocarcinoma: a study of 61 cases and review of the literature, *Mod Pathol*, 13(4):379-88
- Bartek Jiri, Lukas Jiri, Bartkova Jirina, 1999. Perspective: Defects In Cell Cycle Control And Cancer, *J Pathol*, 187:95-99
- Hunter T, Pines J, 1994. Cyclins and cancer. II: Cyclin D and CDK inhibitors come of age, *Cell*, 79: 573-582
- Lamb J, Ramaswamy S, Ford HL, Contreras B, Martinez RV, Kittrell FS, Zahnow CA, Patterson N, Golub TR, Ewen ME, 2003. A mechanism of cyclin D1 action encoded in the patterns of gene expression in human cancer, *Cell*. 114(3): 323-34
- Soslow A. Robert, Shen U-Fu Peter, Chung H. Marilda, Isacson Christina, Baergen N. Rebecca, 2000. Cyclin D1 Expression in High-Grade Endometrial Carcinomas – Association with Histologic Subtype, *Int J Gyn Pathol*, 19:329-334
- Moreno-Bueno G; Rodriguez-Perales S; Sanchez-Estevéz C; Hardisson D; Sarrio D; Prat J; Cigudosa JC; Matias-Guiu X; Palacios J, 2003. Cyclin D1 (CCND1) mutations in endometrial cancer, *Oncogene*, 4; 22(38): 6115-6118
- Cao QJ, Einstein MH, Anderson PS, Runowicz CD, Balan R, Jones JG, 2002. Expression of COX-2, Ki- 67, Cyclin D1, and p21 in Endometrial Endometrioid Carcinomas, *Int J Gynecol Pathol*, 21(2):147-54.
- Alt R. Jodi, Cleveland L. John, Hannink Mark, Diehl Alan J., 2000. Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation, *Genes & Development*, 14:3102-3114
- Kushi AL, Lim P, Aquino-Parsons C, 2002. Markers of Proliferative Activity are Predictors Of Patient Outcome For Low-Grade Endometrioid Adenocarcinoma But Not Papillary Serous Carcinoma of Endometrium, *Mod Pathol*, 15(4): 365-371
- Matias-Guiu X, Catusas L, Bussaglia E, 2001. Molecular pathology of endometrial hyperplasia and carcinoma. *Hum Pathol*, 32:569-77.
- Garcia A.R. Francisco, Davis R. John, Alberts S. David, Liu Yun, Thompson Deborah, Bartels Peter, A 2003. Karyometric Approach to the Characterization of Atypical Endometrial Hyperplasia With and Without Co-occurring Adenocarcinoma, *Anal Quant Cytol Histol*, 25(6): 339-346

1. „Gr. T. Popa” University of Medicine and Pharmacy , Iasi, Romania
  2. The 3<sup>rd</sup> Clinic of Obstetrics and Gynecology Iasi, Romania
  3. „Alexandru Ioan Cuza” University , Iași, Romania
- \* otoma@uaic.ro