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Review Articles

Cyclins in gynecological tumors

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Summary

Cyclins represent a numerous group of proteins which modulate the cell cycle by binding to respective kinases (CDKs) and the formation of active complexes. Cyclin-dependent kinases are present in a cell throughout the cell cycle, activated by partner cyclins they phosphorylate numerous proteins, which are associated with the activation of transcription factors, subsequently involved in the replication of DNA and in modeling of the cell cycle. CDKs may be inhibited by two families of inhibitors: the INK4 family, which compete with cyclin D for binding with CDK4 and CDK6 and CIP/KIP family, which is capable of inhibiting CDK2 and CDK1. In contrast to healthy cells, tumor cells divide beyond any control due to various reasons: an overexpression of cyclins, inactivation of kinase inhibitors or due to the loss of integrity involving factors active in the control points. The cell cycle manifests a variable duration of 20 hours to 5-10 days. In most malignant tumors the cell population doubling time takes more than 50 days. Disturbances in cell cycle control and a disturbed secretion of cyclins, CDKs or CDKI are linked to the development of cancer in several locations.

Key words: Cyclins; Breast cancer; Ovarian cancer; Endometrial cancer; Uterine cervical cancer.

Introduction

Tumor growth reflects several factors, including the cell growth coefficient (the ratio of proliferating cells to resting cells), the cell cycle and coefficient of cell loss, usually due to necrosis or apoptosis, in which the key enzymes are caspases.

The cell cycle consists of four phases; mitotic division (M), post-mitotic G1, DNA replication S, and the pre-mitotic G2 phase. In physiological conditions the cell cycle is mainly controlled by cyclins, cyclin-dependent kinases (CDKs) inhibitors of cyclin-dependent kinases (CDKIs), and numerous transcription factors (E2F family), enzymes activating cell kinases (CAK), and several proteins involved in signaling pathways [1-3].

Cyclins represent a numerous group of proteins which modulate the cell cycle by binding to respective kinases (CDKs) and the formation of active complexes. Several cyclins have been isolated: A to T, but the function of numerous cyclins has not yet been recognized. They are synthesized *de novo* in various phases of the cycle and their variable concentration is linked to the function of CDKs and the progression of the cell cycle. After passage through a specific phase their concentration decreases with the inactivation of CDK and, following ubiquitination, are degraded in proteasomes [1-3].

Cyclin-dependent kinases are present in a cell throughout the cell cycle, activated by partner cyclins they phosphorylate numerous proteins, which is associated with the activation of transcription factors, subsequently involved in the replication of DNA and in modeling of the cell cycle. At least nine structurally linked CDKs have been identified (CDK1-CDK9), though not all of them have been ascribed a specific function in the cell cycle. CDKs may be inhibited by two families of inhibitors: the INK4 family, including $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$, which compete with cyclin D for binding with CDK4 and CDK6 and CIP/KIP family, including $p21^{CIP1}$ (or $p21^{WAF1}$), $p27^{KIP1}$ and $p57^{KIP2}$, which is capable of inhibiting CDK2 and CDK1 [1, 3-5].

All three principal groups of cell cycle controllers are active in specific phases of the cell cycle. In the G1 phase, lasting from the end of mitosis to the beginning of the S phase, the longest phase of the cell cycle lasting from a few to more than ten hours, the priming cyclins involve cyclin D (or its isoforms D1-D3), forming complexes with CDK4 and CDK6, and cyclin E, forming a complex with CDK2. This complex, phosphorylated by cyclins D and E and respective CDKs (CDK4 and CDK6) detaches from the transcription factor E2F-DP, which initiates the transcription of multiple genes required for the cell to enter phase S. The passage from phase G1 to phase S is controlled by pRb (a product of the suppressor *Rb* gene). This control point involves also the function of p53; in cases of DNA damage

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activation of p53 follows, with a subsequent increase in concentration of $p21^{CIP1}$, which binds to CDK2 and CDK4 inhibiting their action and blocking cell cycle at the G1 phase [1, 4, 6].

At the phase of synthesis cyclins A, B, and D are active, binding to appropriate cyclin-dependent kinases (CDK1, CDK2, CDK4, and CDK6). In addition, in this phase, CDK7 and CDK9 are active, manifesting a double function: they activate all CDKs and stimulate transcription. The duration of the phase is around seven hours [1, 2]. The G2 phase is linked to an increase in concentration of cyclin B, which following complexing with CDK1 represents the main component of mitosis promoting factor (MPF). This factor stimulates the passage of the cell from the G2 to M phase [7].

The control point of G2/M involves the control of DNA integrity before the cell enters phase M. The proteins p53 (again) and $p21^{WAF1}$ [7, 8] are active in the control.

In phase M, lasting around one hour, cyclins B and D are active, forming active complexes with the respective kinases and, as a result of complex molecular processes, two descendant cells are formed while cyclins undergo degradation in the proteasomes. Following mitosis, the descendant cell either enters the G1 phase again or the resting G0 phase, from which it may re-enter the G1 phase or die [1-3].

In contrast to healthy cells, tumor cells divide beyond any control due to various reasons: an overexpression of cyclins, inactivation of kinase inhibitors or due to the loss of integrity involving factors active in the control points. The cell cycle manifests a variable duration of 20 hours to 5-10 days. In most malignant tumors the cell population doubling time takes more than 50 days [9].

Disturbances in cell cycle control and a disturbed secretion of cyclins, CDKs or CDKI are linked to the development of cancer in several locations [2, 4, 10, 11].

Breast cancer

A disturbed control of the cell cycle plays an important role in the development of breast cancer. Nin *et al.* [11] using immunohistochemistry examined the concentration of cyclin E in breast carcinoma tissue; the control group involved benign fibro-adenomas. An overexpression of cyclin E in breast cancer was found to be linked to the expression of estrogen receptors and to metastases of the cancer to lymph nodes. The investigators confirmed this link on established cell lines of breast cancer, MCF-7. The concentration of cyclin E increased following supplementation with 17β-estradiol and decreased after the application of tamoxifen. This indicated that the estrogen receptor played a critical role in the control of cyclin E production, the overexpression of which may provide a marker of poor prognosis. Wei *et al.* [12] provided evidence of the effects of receptor status on the expression of cyclin D1b: the overexpression of cyclin D1b was linked to a triple-negative cancer and poor prognosis in patients. Such a significance of cyclin overexpression was confirmed using an animal model: silencing of the gene coding for cyclin D1b using D1bsiRNA inhibited growth of the tumor and amplified the effects of doxorubicin, pointing to the potential for therapy targeted at the gene in breast cancer with an overexpression of cyclin D1b.

Mayer [10] expressed the opinion that the dysregulated cell cycle opening potential for the development of cancer and its metastases can be subjected to control again in a therapy-attractive manner. However, the selective inhibition of CDK4 and CDK6 using palbociclib relies on interaction of the kinases with cyclin D1. The results of investigations on the drug may provide a new standard of therapy in breast cancer.

Morikawa and Henry [13] corroborated the targeted action of palbociclib. In breast cancers showing positivity for estrogen receptors and negative for HER2, its action depends on the reduction of Rb phosphorylation, linked to its activity and the blocking of the cell cycle through the inhibition of cyclin D-associated CDK4 and CDK6. Other studies showed that treatment using palbociclib in 37 patients with amplified cyclin D1 gene (CCND1), expression of receptors for estrogens, active Rb and negative HER2 significantly elongated progression-free survival (PFS) [14].

Cyclin A represents another cyclin engaged in the development of various cancers, including breast cancer [9, 15]. High expression of the cyclin estimated immunohistochemically in 283 women with metastatic breast cancer correlated with an abbreviated time to its first relapse and the abbreviated duration of survival following the diagnosis. The authors thought that cyclin A provided a good marker of tumor proliferation and a prognostic marker of patient's survival [15].

Ovarian cancer

Interesting results of studies on cyclin E were presented by Karst *et al.* [16]. They are related to etiopathogenesis of ovarian serous G3 cancers, supposedly originating from transformed oviduct cells. Since genomic analyses identified the amplification of the cyclin E1 gene as a possible stimulator of oncogenesis in high grade (G3) serous ovarian cancer, the authors examined the expression of cyclin E1 in non-transformed cells of the oviduct, in early and advanced oviduct tumors. The expression of cyclin E proved to be more pronounced in cancers. The authors expressed the opinion that the loss of control over cyclin E production stimulated malignant transformation in oviduct secretory cells, the site of origin for serous ovarian cancers.

In numerous studies a relationship was documented between the expression of cyclin D1 and the course of ovarian cancer [17, 18]. In immunohistochemical investigations by Turan *et al.* [17], the expression of a few molecular markers (HER-2/neu, survivin, cyclin D1) was studied in benign cysts, borderline tumors. and serous ovarian can-

cers. Immunoreactivity of HER-2/neu and survivin manifested increasing values upon passage from benign cysts to cancers. In malignant ovarian tumors a high expression of cyclin D1 was disclosed (in 95.6% of the patients), a lower one in borderline tumors (85.7%), and the lowest was recorded in benign lesions (48%). Cyclin D1 was found to provide a marker in serous ovarian cancer, similarly to HER2/neu and survivin. The expression of cyclin D1 was found to manifest a correlation with degree of cancer malignancy. In studies by Lin and Yu [18], a relationship was suggested between the expression of cyclin D1 and that of nucleostymin, undetectable in healthy ovarian tissue. Similarly to cyclin D1, nucleostymin expression was linked to a G1/S restriction point. In the opinion of the authors, through cyclin D1, nucleostymin affects the control of the cell cycle between the G1 and S phases. Similarly to Turan *et al.* [17], Lin and Yu [18] detected the positive expression of cyclin D1 in 90% of malignant tumors, 80% of borderline cancers and 20% of benign tumors. The effect of nucleostymin on the expression of cyclin D1 requires further investigation.

Controversial results of studies were obtained in 172 cases of ovarian cancer at various stages of advancement (II to IV) and the expression of CCNE1 gene coding for cyclin E1 [19]. A high expression of the gene was found to exhibit a significant correlation with longer survival. The authors expressed the opinion that a high expression of cyclin E1 represents a significant and an independent predictive index of longer survival, particularly at FIGO Stages III and IV.

Wang *et al.* [20] examined the expression of β-catenin, associated with cyclin D1 in 60 women with ovarian cancer. β-catenin expression was detected more frequently in patients with FIGO Grades III and IV than in those with FIGO Grades I and II ($p = 0.003$). The authors found that β-catenin and cyclin D1 may provide markers of poor prognosis in the cancer.

Another cyclin, cyclin A and factor YB1 (Y-BOX-binding protein1) are engaged in transcription, translation, tumor growth, invasiveness. and resistance to treatment [21]. In women burdened with ovarian cancer, a higher expression of both cyclin A and YB1 in immunohistochemical studies were found to be more pronounced in advanced cancers (FIGO III/IV), cancers manifesting low differentiation (G3) and in cancers which after debulking left remnants of > 1 cm. Thus, the overexpression of cyclin A was linked to more pronounced tumor aggression and poor prognosis.

Endometrial cancer

In order to evaluate the role of cyclin D overexpression in the development of endometrial cancer, the expression was evaluated in hypertrophies (simple and complex) and in cancers. Immunohistochemical analysis of cyclin D1 in precancerous conditions and in endometrial cancer showed that its expression was manifested in less than 40% of hypertrophic lesions and endometrial cancers. Statistical analysis demonstrated that patients with a high expression of cyclin D1 carried a metastatic disease. In the opinion of the authors, cyclin provided a key marker of metastatic endometrial cancer even if it could not distinguish between benign and malignant lesions [22].

Zapiecki *et al.* [23] estimated the nuclear expression of cyclins D1 and E in two histological types of endometrial cancer: clear cell carcinoma and adenosquamous carcinoma. The expression of cyclin D1 was typical for endometrial cancer with squamocellular differentiation while its overexpression was linked to poor prognosis. Cyclin E manifested overexpression in clear cell carcinoma and it was linked to the shorter survival of the patients. Studies in 211 patients with endometrioidal cancer of the endometrium evaluated the overexpression of cyclin E using an immunohistochemical approach. The analysis showed that the respective correlation was related to a degree of differentiation (G), and not to FIGO advancement, as demonstrated in other studies, or to the invasion of myometrium [24]. The same authors also examined the expression of cyclins A and B in the group of endometrial cancers [8, 25]. Since cyclin A participates in restriction points G1/S and G2/M, and cyclin B activates CDK1, significant for the G2/M passage, their expression may be linked to a loss of control over the cell cycle. The authors showed that the overexpression of cyclin A correlated with the degree of histological differentiation G and the grade of advancement according to FIGO and represented an independent prognostic index in endometrioidal cancer of endometrium. The expression of cyclin B also correlated with the parameters (G and the grade of advancement). The authors expected that the expression of cyclin may carry also a prognostic significance, but this requires further investigation.

Cancer of the uterine cervix

Highly oncogenic HPV are strictly linked to the development of precancerous conditions and cancer of the uterine cervix through the interaction of E6 and R7 oncoproteins with controllers of the cell cycle, such as p53 and Rb. The expression of p53, p21, and p16 (CDK inhibiting proteins), Ki-67 and cyclin D1, as well as the presence of HPV were examined in precancerous conditions and in cancer of the uterine cervix. Highly oncogenic HPV were detected in 96.3% of the cases. A significantly augmented expression of p16, p21, and p53 was detected in precancerous conditions up to the cancer while the expression of cyclin D1 proved to undergo a marked reduction ($p < 0.001$). The authors thought that the estimation of factors involved in the control of the cell cycle, p16, and cyclin D1 in particular may provide additional markers useful in the differentiation of precancerous conditions from cancer of the uterine cervix [26].

Wang *et al.* [27] conducted studies on cell lines of cancer

in the uterine cervix with a stable expression of cyclin D1. They proved that an increase in the concentration of cyclin D1b (isoform of cyclin D1) blocked the cell cycle at the G0/G1 phase and induced apoptosis, blocking in this way cell proliferation *in vivo*. Their studies pointed to the antitumorous effect of cyclin D1b in cancer of the uterine cervix; cyclin may provide a therapeutic target in the cancer.

Detailed studies on cyclin D1 (G870A) polymorphism were subjected to analysis of PubMed and Embase databases. The meta-analysis included 2,864 cases of cancer of the uterine cervix and 3,898 cases of the control group. No significant correlation could be disclosed between polymorphism of cyclin D1 and the risk of cancer development in the uterine cervix. Asiatic and Caucasian ethnic subgroups were also examined. Such meta-analysis of cyclin D1 (G870A) polymorphism could not identify grounds for it to be linked with a genetic propensity toward development of cancer in the uterine cervix.

In summary beyond doubt, cyclins as controllers of the cell cycle in conditions of its derangement are involved in the development of cancers in many locations; as demonstrated they may provide prognostic markers of the disease as well as a promising therapeutic target, similarly to the examined tyrosine kinase inhibitors, of which several have already found a therapeutic role.

References

[1] Schwartz G.K., Shah M.A.: "Targeting the cell cycle: a new approach to cancer therapy". J. *Clin. Oncol.*, 2005, *23*, 9408.

[2] Gallorini M., Cataldi A., di Giacomo V.: "Cyclin-dependent kinase modulators and cancer therapy". *BioDrugs*, 2012, *26*, 377.

[3] Satyanarayana A., Kaldis P.: "Mammalian cell-cycle regulation: several Cdks, numerous cyclins and diverse compensatory mechanisms". *Oncogene*, 2009, *28*, 2925.

[4] Sampath D., Plunkett W.: "Projektowanie nowych terapii przeciwnowotworowych ukierunkowanych na punkty kontrolne cyklu komórkowego". *Curr. Opin. Oncol.*, 2002, *2*, 4.

[5] Besson A., Dowdy S.F., Roberts J.M.: "CDK inhibitors: cell cycle regulators and beyond". *Dev. Cell.*, 2008, *14*, 159.

[6] Potemski P., Kordek R.: "Znaczenie prognostyczne cykliny E w raku piersi. Onkologia w Praktyce Klinicznej". *Via Medico*, 2005, *2*, 96.

[7] Zdrojewicz Z., Belowska-Bień K.: "Cyklina - E - nowy marker w onkologii". *Adv. Clin. Exp. Med.*, 2004, *13*, 1023.

[8] Santala S., Talvensaari-Mattila A., Soini Y., Santala M.: "Prognostic value of cyclin B in endometrial endometrioid adenocarcinoma". *Tumor Biol.*, 2015, *36*, 953.

[9] Yam CH., Fung T.K., Poon R.Y.: "Cyclin A in cell cycle control and cancer". *Cell Mol. Life Sci.*, 2002, *59*, 1317.

[10] Mayer E.L.: "Targeting breast cancer with CDK inhibitors". *Curr. Oncol. Rep.*, 2015, *17*, 443.

[11] Niu D., Wang G., Wang X.: "Up-regulation of cyclin E in breast cancer via estrogen receptor pathway". *Int. J. Clin. Exp. Med.*, 2015, *8*, 910.

[12] Wei M., Zhu L., Li Y., Chen W., Han B., Wang Z., et al.: "Knocking down cyclin D1b inhibits breast cancer cell growth and suppresses tumor development in a breast cancer model". *Cancer Sci.*, 2011, *102*, 1537.

[13] Morikawa A., Henry N.L.: "Palbociclib for the treatment of estrogen receptor-positive, HER2-negative metastatic breast cancer". *Clin. Cancer Res.*, 2015, *21*, 3591.

[14] DeMichele A., Clark A., Tan KS., Heitjan D., Gramlich K., Gallagher M., *et al.*: "CDK 4/6 inhibitor Palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety and predictive biomarker assessment". *Clin. Cancer Res.*, 2015, *21*, 995.

[15] Poikonen P., Sjöström J., Amini RM., Villman K., Ahlgren J., Blomqvist C.: "Cyclin A as a marker for prognosis and chemotherapy response in advanced breast cancer". *Br. J. Cancer*, 2005, *93*, 515.

[16] Karst A.M., Jones P.M., Vena N., Ligon A.H., Liu J.F., Hirsch M.S., et al.: "Cyclin E1 deregulation occurs early in secretory cell transformation to promote formation of fallopian tube-derived high-grade serous ovarian cancers". *Clin. Res.*, 2014, *74*, 1141.

[17] Turan G., Usta C.S., Usta A., Kanter M., Tavli L., Karacan M., *et al.*: "The expression of HER-2/neu (c-erbB2), surviving and cycline D1 in serous ovarian neoplasms: their correlation with clinicopathological variables". *J. Mol. Histol.*, 2014, *45*, 679.

[18] Lin S., Yu M.S.: "Clinical significance of nucleostimin expression and its correlation with cyclin D1 expression in malignant ovarian tumors". *Int. J. Gynecol. Cancer*, 2011, *21*, 1166.

[19] Pils D., Bachmayr-Heyda A., Auer K., Svoboda M., Auner V., Hager G., *et al.*: "Cyclin E1 (CCNE1) as independent positive prognostic factor in advanced stage serous ovarian cancer patients - A study of the OVCAD consortium". *Eur. J. Cancer*, 2014, *50*, 99.

[20] Wang H., Wang H., Makki MS., Wen J., Dai Y., Shi Q.: "Overexpression of β-catenin and cyclinD1 predicts a poor prognosis in ovarian serous carcinomas". *Int. J. Clin. Exp. Pathol.*, 2013, *7*, 264.

[21] Cybulski M., Jarosz B., Nowakowski A., Jeleniewicz W., Kutarska E., Bednarek W., Stepulak A.: "Cyclin A correlates with YB1, progression and resistance to chemotherapy in human epithelial ovarian cancer". *Anticancer Res.*, 2015, *35*, 1715.

[22] Liang S., Mu K., Wang Y., Zhou Z., Zhang J., Sheng Y., Zhang T.: "Cyclin D1, a prominent prognostic marker for endometrial diseases". *Diagn. Pathol.*, 2013, *15*, 138.

[23] Zapiecki K., Manahan K.J., Miller G.A., Geisler J.P.: "Cyclin E is overexpressed by clear cell carcinomas of the endometrium and is a prognostic indicator of survival". *Eur. J. Gynaecol. Oncol.*, 2015, *36*, 114.

[24] Santala S., Talvensaari-Mattila A., Soini Y., Santala M.: "Cyclin E expression correlates with cancer-specific survival in endometrial endometrioid adenocarcinoma". *Anticancer Res.*, 2015, *35*, 3393.

[25] Santala S., Talvensaari-Mattila A., Soini Y., Honkavuori-Toivola M., Santala M.: "High expression of cyclin A is associated with poor prognosis in endometrial endometrioid adenocarcinoma". *Tumor Biol.*, 2014, *35*, 5395.

[26] Portari E.A., Russomano F.B., de Camargo M.J., Machado Gayer C.R., da Rocha Guillobel H.C., Santos-Rebouças C.B., Brito Macedo J.M.: "Immunohistochemical expression of cyclin D1, p16Ink4a, p21WAF1, and Ki-67 correlates with the severity of cervical neoplasia". *Int. J. Gynecol. Pathol.*, 2013, *32*, 501.

[27] Wang N., Wei H., Yin D., Lu Y., Zhang Y., Jiang D., et al.: "Cyclin D1b overexpression inhibits cell proliferation and induces cell apoptosis in cervical cancer cells in vitro and in vivo". *Int. J. Clin. Exp. Pathol.*, 2014, *7*, 4016.

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