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THE ROLE OF CELL CYCLE GENES IN THE DIAGNOSIS AND THERAPY OF OVARIAN CANCER

Alessandro Bovicelli¹, Giuseppina D'Andrilli², Antonio Giordano²,
Domenico De Aloisio¹

Clinica Ostetrica e Ginecologia e Fisiopatologia della Terza Eta'

Indirizzo per corrispondenza: Dott. Alessandro Bovicelli, M.D., Ph.D.

Department of Obstetrics and Gynecology, Sant'Orsola Hospital, University of Bologna
Via Massarenti 13, 40138 Bologna, Italy

tel: +39 051 399520; fax: +39 051 304751; e-mail: alessandro.bovicelli@unibo.it

ABSTRACT

Human malignant tumors are characterized by abnormal proliferation resulting from alterations in cell cycle regulatory mechanisms. The regulatory pathways controlling cell cycle phases include several oncogenes and tumor suppressor genes which display a range of abnormalities with potential usefulness as markers of evolution or treatment response in ovarian cancer. This review summarizes the current knowledge about these aberrations in malignant tumors of the ovary. We sought to focalize our attention on the genes involved in the regulation of the G1 phase and the G1-S transition of the cell cycle, two crucial steps in the cell cycle control.

Key words: *cell cycle deregulation, gynecological malignancy, cyclins, cancer therapy, tumor suppressor genes.*

RIASSUNTO

I tumori maligni umani sono caratterizzati da una abnorme proliferazione che risulta dalla alterazione dei meccanismi regolatori del ciclo cellulare. La sequenza regolatoria che controlla le fasi del ciclo cellulare include alcuni oncogeni e geni soppressori dei tumori i quali mostrano una gamma di anomalie con una potenziale utilità come marcatori della progressione tumorale o della risposta al trattamento nel tumore ovarico. Questa review riassume la conoscenza attuale riguardo a queste aberrazioni dei tumori maligni dell'ovaio. I geni regolatori del ciclo cellulare sono stati suddivisi in quattro sottogruppi, in base al loro ruolo predominante, in una fase specifica o durante la transizione fra due fasi del ciclo cellulare.

Parole chiave: *deregolazione del ciclo cellulare; patologia ginecologica.*

INTRODUCTION

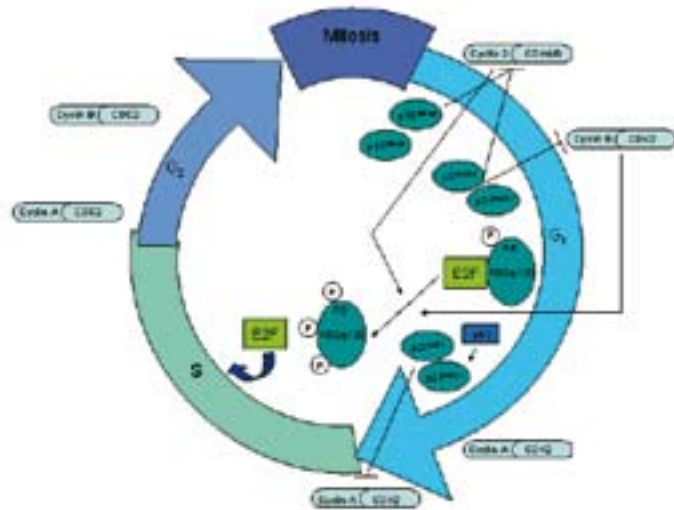
Cancer is frequently considered to be a disease of the cell cycle; alterations in different families of cell cycle regulators cooperate in tumor development. Molecular analysis of human tumors has shown that cell cycle regulators are frequently mutated in human neoplasms, which underscores how important the maintenance of cell cycle commitment is in the prevention of human cancer. Mammalian cell division is precisely regulated in a timely manner by a family of protein kinases, the cyclin-dependent kinases (CDKs), which is a group of serine/threonine kinases that form active heterodimeric complexes following binding to cyclins, their regulatory subunits. Regulation of CDK activity occurs at multiple levels, including cyclin synthesis and degradation, phosphorylation and dephosphorylation, CDK inhibitor (CKI) protein synthesis, binding and degradation, and subcellular localization. Orderly progression through the cell cycle involves coordinated activation of the CDK protein by binding to the cyclin partner. A succession of kinases (CDK4, CDK6,

CDK2, and CDC2) are expressed along with a succession of cyclins (D, E, A, and B) as cells go from G₁ to S to G₂ to M phase (Figure1).

Different CDK-cyclin complexes operate during different phases of the cell cycle. Active CDK-cyclin complexes phosphorylate target substrates, including members of the "pocket protein" family (pRb, p107 and pRb2/p130) (1-3). G₁-S transition in normal cells requires phosphorylation of the retinoblastoma protein Rb and the related proteins Rb2/p130 and p107 by CDKs, which causes the release of E2F transcription factors controlling various genes required for DNA synthesis and cell cycle control.

Endogenous inhibition of CDKs is also caused by two families of regulatory proteins induced under mitogenic stimuli: the INK4 family, comprising p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, and p19^{INK4d}, which specifically inhibit CDK4 and CDK6 (4), and the CIP/KIP family including p21^{CIP1/WAF1}, p27^{KIP1} and p57^{KIP2}, which cause a broader range of inhibition and act in a concentration-dependent manner (5). All CKIs cause G₁ arrest when overexpressed in cells by association and inhibition of the CDKs. INK4 proteins dissociate cyclin D/CDK complexes and redistribute

Figure 1) Schematic model of the normal mammalian cell cycle. Negative regulators of the cell cycle are indicated in dark blue, whereas positive controllers are shown in light blue. G₁-S transition in normal cells requires phosphorylation of the retinoblastoma proteins by CDKs, which causes the release of E2F transcription factors controlling various genes required for DNA synthesis during S phase. The CDK inhibitors p21^{WAF1} and p27^{KIP1} act by binding to cyclin-CDK2 complexes to inhibit their catalytic activity and induce cell cycle arrest, whereas p16^{INK4a} inhibits CDK4/6. Wild-type p53 activates the transcription of p21 gene.



the CIP/KIP proteins to CDK2, producing a double inhibition. At low concentrations, CIP/KIP family proteins enhance CDK4 association with cyclin D, increasing the activity of the complex, whereas at high concentrations they inhibit kinase activity, presumably by increasing the stoichiometry in the CDK complexes (6). The best studied events of the cell cycle are the G₁ phase preceding the DNA synthesis (S) phase and the mechanism that drives the cell across the restriction (R) point in late G₁, which is crucial for the cell's destiny towards division, differentiation, senescence or apoptosis. Several studies suggest that traversal of the restriction point within the G₁ phase is the key event in cell cycle regulation, and that the rest of cell cycle progression occurs almost automatically once the R point has been overcome (7). Several proteins can inhibit the cell cycle in G₁ phase; if DNA damage occurs, p53 accumulates in the cell and induces the p21-mediated inhibition of cyclinD/CDK. The frequent loss of G₁ regulation in human cancer has revealed targets for possible therapeutic intervention. In contrast to G₁ regulators, less is known about the genes which regulate the S, G₂ and M phases of the cell cycle like cyclin A- and cyclin B-kinase complexes and their inhibitors. The significance of cell-cycle regulatory genes in carcinogenesis is underlined by the fact that most of them have been identified as proto-oncogenes or tumor suppressor genes.

Ovarian cancer: background

Ovarian cancer remains a highly lethal disease. In developed countries, ovarian cancer accounts for more deaths than all other gynecological malignancies combined; it occurs in 1 out of 57 women in the United States (Cancer Facts and Figures 2005). As the result of advances in surgical management and chemotherapeutic options over the last three decades, the median survival for ovarian cancer patients has improved. However, overall survival has not been significantly altered. Although susceptibility genes such as BRCA1 and BRCA2 have been identified, a majority of ovarian cancers occur sporadically without known risk factors. In addition, most patients present with advanced disease for which highly effective curative

therapy is currently unavailable. Depending upon the cell of origin, ovarian neoplasms can be divided into epithelial tumors which account for 90% of the malignant tumors, sex chord-stromal tumors and germ cell tumors. The epithelial tumors are further divided into histological subgroups with different malignant potential namely endometrioid, mucinous, serous, clear cell and undifferentiated carcinomas.

In follow-up studies, mucinous and endometrioid carcinomas have a less aggressive behavior and a better overall survival than serous tumors. A characteristic of serous, mucinous and endometrioid ovarian carcinomas is the low malignant potential (LMP) or the presence of borderline tumors having a low risk of invasion. Hopefully a better understanding of the molecular mechanisms underlying the tumorigenic process of ovarian carcinoma will lead to earlier diagnosis, novel therapies and ultimately better outcomes.

Cell cycle G₁ phase regulators

D-type cyclins are transcribed in the G₁ phase of the cell cycle. The isoforms D1, D2, and D3 are functionally equivalent, and are expressed in a tissue-specific manner. CDK4 and CDK6 are activated by D cyclins to phosphorylate the retinoblastoma protein pRb, a known cell proliferation regulator. The members of the INK4 family exert their inhibitory activity by binding to the CDK4 and CDK6 kinases and preventing their association with D-type cyclins.

Cyclin D1 gene

In contrast to other tumor types, the cyclin D1 gene (CCND1) is rarely amplified in ovarian carcinomas. In 65 specimens analyzed by Masciullo et al., the frequency of the overexpression of the gene was estimated to be 18%, but none of the tumors showed amplification of cyclin D1. No difference in cyclin D1 mRNA levels was found between primary and recurrent disease. A statistically significant correlation was found between elevated levels of cyclin D1 and well to moderately differentiated grade (G1-G2) (p= 0.005), but no association with clinical outcome was found (8).

Overexpression of cyclin D1 was also observed by Dhar et al. in 89% of 81 epithelial ovarian tumor sections in both borderline and invasive tumors. There was no association between protein overexpression and tumor stage or grade of differentiation. Furthermore, no correlation between cyclin D1 expression and clinical outcome was observed. Amplification of the cyclin D1 gene was detected in only one of a subset of 29 tumors showing overexpression of cyclin D1 protein. The authors concluded that deregulation of CCND1 expression leading to both cytoplasmic and nuclear protein localization is a frequent event in ovarian cancer and occurs mainly in the absence of gene amplification (9).

Barbieri et al. defined the pattern of cyclin D1 expression in the development of ovarian cancer in 55 cases of benign ovarian tumors, 12 borderline cases and 37 ovarian carcinomas. A statistically significant increase in median cyclin D1 values was observed from benign, to borderline, to malignant, to recurrent tumors. The authors found a significant relationship between cyclin D1 expression and progression-free survival (P=0.031) (10).

Cyclin D1 was found to be overexpressed mainly in borderline and low-grade ovarian tumors, in contrast with high-grade tumors, by Sui et al. Within the malignant tumor group, the authors found an association between higher cyclin D1 expression and a well-differentiated phenotype (G1, G2). The reason why cyclin D1 was decreased in the high-grade (G2 and G3) tumors is not clear. Some possible explanations can be made. First, there may be multiple pathways in the development of ovarian tumors. The authors hypothesized that cyclin E rather than cyclin D1 plays a predominant regulatory role in the progression of ovarian carcinomas. Second, the tumor cellular origins of borderline

and low-grade tumors may be different from those of high-grade tumors (11). These observations are similar to those obtained with breast cancer studies, where cyclin D1 overexpression is associated with a favorable prognosis (12).

Cyclin D2 gene

Similar to cyclin D1, the cyclin D2 gene is only sporadically amplified in ovarian tumors. Courjal et al. investigated cyclin D2 expression in 237 ovarian tumors. Cyclin D2 only on rare occasions, showed increased DNA copy numbers and were never found to be overexpressed at the RNA level (13).

Expression studies on various tumor types have shown that more than 80% of the granulosa cell tumors, but only single epithelial tumors express high levels of cyclin D2 mRNA (14).

Milde-Langosch et al. detected cyclin D2 protein expression in all analyzed granulosa cell tumors (n=7) but in only 23% of 93 ovarian epithelial carcinomas (15,16). Thus, cyclin D2 overexpression is characteristic of a single histological tumor type which accounts for 6% of all ovarian malignancies.

CDK4 gene

Overexpression of CDK4 has been found in 14-15% of a relatively large number of ovarian tumors on mRNA and protein levels (8) although gene amplification has not been demonstrated so far (8). CDK4 status has not been associated with clinical outcome (17).

CDK4 overexpression has been reported to be associated with an increased expression of cyclin D1 (8) and low p16 expression (18). Sui et al. described a significant increase of CDK4 activity in malignant ovarian tumors in contrast with benign tumors ($p < 0.01$), suggesting that CDK4 activity may play an important role in ovarian carcinogenesis (18).

p16^{INK4a} gene

p16 has been widely investigated in ovarian tumors on the DNA, RNA, and protein levels. p16 deletion occurred at a rate of 50% in 12 ovarian cancer cell lines analyzed by Fang et al. (19) in spite of the lack of such a frequency of mutation in primary ovarian cancer cells (20). They also observed that p16 expression induced transcriptional downregulation of the RB gene (19). Ovarian cancer cell lines coexpressing p16 and RB are insensitive to p16 overexpression, suggesting that tumors that express both genes may be unresponsive to p16 gene therapy (21). Loss of expression of the p16 tumor suppressor occurs more often in ovarian cancers lacking p53 mutations (22) consistently with the paradigm that inactivation of p53 is less important in ovarian carcinogenesis when another G₁ regulatory gene has already been inactivated. The growth inhibitory effect of p16 has been confirmed in a study carried out with the two ovarian cancer cell lines SKOV3 and OVCA-420 (23). In SKOV3 cells, G₁-arrest induced by p16-transduction prevents paclitaxel- and vindesine-induced cell death (24). High-level p16 expression was observed in serous and endometrioid phenotypes, with a positive relation to high levels of both cell proliferation and p53 abnormalities. None of 131 cases showed a methylation status of the p16 gene promoter (25). Two different studies also revealed no evidence of methylation and low levels of mutations (26, 27). However there are some data supporting p16 promoter hypermethylation as a mechanism underlying the downregulation of the gene. Milde-Langosch et al. found hypermethylation in 12/19 negative cases most of them mucinous and endometrioid carcinomas (28). Suh et al. demonstrated that both promoter methylation and aberrant mRNA processing may interfere with p16 expression in ovarian tumors (29). Kudoh et al. found homozygous deletion in 18% of 45 patients analyzed and suggest that deletion of p16 is a potential indicator for poor

chemotherapy response and adverse prognosis in ovarian cancer patients (30). In a study carried out on 190 epithelial ovarian tumors the authors found that a high number of p16 positive tumor cells was associated with advanced stage and grade, and with poor prognosis (31). On the other hand, in a recent study, a significant influence of p16 expression on overall survival was not confirmed (15). Higher levels of p16 are expressed in retinoic acid (RA)-sensitive CAOV3 cells compared to RA-resistant SKOV3 cells (32).

p15^{INK4b} gene

The p15 gene, which is located on 9p, contains sequences highly homologous to exon 2 of p16. The p15 gene also inhibits both CDK4 and CDK6 kinase activities (33,34). Little is known about the potential role of this gene in ovarian epithelial tumors. Ichikawa et al. investigated the involvement of p15 inactivation in ovarian tumorigenesis with 49 primary ovarian tumors and 6 ovarian cancer cell lines. Homozygous deletion was found in 10% of primary tumors but mutation of p15 was not detected in any sample. Alterations in p15 were observed in serous, endometrioid and clear cell but not in mucinous carcinomas suggesting that inactivation of p15 may be the histological type-specific event in ovarian tumorigenesis (35). In contrast to this study, among 70 ovarian epithelial tumors, a p15 mutation occurred in only a single ovarian tumor and homozygous deletion of the p15 gene was observed in only one additional case, suggesting that the p15 gene may not play an important role in ovarian carcinogenesis (36). Kudoh et al. found homozygous deletion of p15 in 33% of 45 cases, moreover the deletion of the gene was a potential indicator for poor chemotherapy response and a significant poor prognostic factor in advanced ovarian cancer (30).

A recent study has shown that homozygous deletion of p15 may account for TGF β resistance in some populations of ovarian cancer cells (37). p15 was found hypermethylated in a recent study (38) and promoter hypermethylation of p15 was observed in 30,8 % of 52 ovarian cancer cases (39).

Cell cycle G₁-S transition regulators

Genetic analysis of human tumors has revealed that some of the molecules most often altered in cancer are those involved in the control of the G₁-S transition of the cell cycle, a time when cells become committed to a new round of cell division. During the G₁-S transition, the cyclinE/CDK2 and cyclinD/CDK4 complexes promote progression and are each inhibited by the associated CDK inhibitor p27. If DNA damage occurs, p53 accumulates in the cells and induces the p21-mediated inhibition of cyclinD/CDK. The transition to S phase is triggered by the activation of the cyclinD/CDK complex, which phosphorylates pRb.

Cyclin E gene

Marone et al. hypothesized that cyclin E and CDK2 are, in part co-regulated and may have a role in ovarian tumor development after having found that cyclin E and CDK2 are regulated in ovarian tumors by gene amplification and at the level of RNA transcriptional control (40).

Clear cell carcinoma revealed significantly increased cyclin E associated with an increase in p21, compared to the other histological subtypes (41). Sui et al. found higher levels of cyclin E expression in ovarian carcinomas with respect to benign tumors, gradually increasing from benign (9.1%) to borderline (47.8%) to malignant ovarian tumors (70.2%) ($P < 0.0001$). This finding indicated that cyclin E overexpression is closely associated with the malignant biological feature of ovarian tumors. In addition, cyclin E overexpression correlated with advanced clinical stage and the presence of ascites.

High mortality risk was associated with cyclin E overexpression (RR, 2.02; $P = 0.034$) suggesting that increased cyclin E expression not

only contributed to the development of ovarian malignancy, but also correlated with the poor prognosis of ovarian carcinoma patients. The authors found that patients with p27(-) cyclin E (++) / CDK2 (++) had an almost three-fold higher relative risk of mortality (RR, 2.91; $P=0.0001$) which was independently associated with poor overall survival ($P=0.035$)⁴² The expression of cyclin E and CDK2 gradually increased from benign to borderline to malignant tumors in a study carried out on 103 cases, suggesting that overexpression of cyclin E or CDK2 was significantly associated with malignancy in ovarian tumors (42). The prognostic relevance of cyclin E was also evaluated by Farley et al. in 139 cases of primary AOC (Advanced Ovarian Cancer). High cyclin E was associated with worse survival only in the subgroup of women who received the combination of cisplatin and taxol. This may be influenced by the superior efficacy of a taxol-containing regimen compared to the cytoxan regimen. Conversely, cyclin E expression may modulate the cell's sensitivity to taxol. Cyclin E associated CDK activity could be an important molecular complex for targeted therapy. It is interesting to hypothesize that effective inhibition of cyclin E may enhance ovarian cancer sensitivity to cisplatin and taxol in combination (43).

RB gene

Alterations in the retinoblastoma gene (RB) are common in human neoplasia. Among the RB family members, RB is the most investigated gene in ovarian cancer disease. The RB gene was found to be abnormal in four ovarian cancer cell lines of six analyzed, suggesting a role for this gene in the carcinogenesis of some human ovarian tumors at the point of RB gene inactivation (44). Dong et al. showed that most of the malignant ovarian tumors among 125 specimens (71%) had a strong pRb expression compared to normal ovaries in which the protein is hardly detectable. Reduced pRb expression was the significant predictor for poor prognosis in stage I patients. Moreover the relationship between the expression of pRb and p16 depended on tumor stage: in stage I tumors, the authors found an inverse correlation, whereas most advanced tumors showed a direct correlation between pRb and p16 (31). It is also reported that Rb protein and mRNA are expressed at higher levels in cell lines lacking p16 than in those with normal p16 (19). These findings are in accordance with the knowledge that RB and p16 tumor suppressor genes function in the same pathway of cell cycle control. Investigations regarding the pRb/cyclin D1/p16 pathway showed that coexpression of pRb, p16 and cyclin D1 is present in 82% of ovarian cancer tissues and cell lines, suggesting that defects in the pRb/cyclin D1/p16 pathway, other than the loss of pRb or p16, may play a major role in the development of ovarian cancer (21). Nieman et al. showed that for most ovarian carcinomas RB alteration is not necessary for the development of a malignant phenotype, and RB mutation, when it does occur, may represent a sporadic event in ovarian carcinogenesis (45). Ovarian cancer cells with wild-type pRb are sensitive to BRCA1-induced growth suppression suggesting that pRb is involved in the growth suppressor function of BRCA1 (46). Diminished pRb levels are related to several clinicopathological indicators of aggressiveness in ovarian adenocarcinomas such as increasing grade, advancing stage and bulk residual disease (47). Critical interactions between p53 and pRb pathways in ovarian carcinoma pathogenesis are emerging from a recent study by Flesken-Nikitin et al., who provided direct genetic evidence that defects in p53- and pRb-mediated pathways cooperate in ovarian carcinogenesis (48).

RB2/p130 gene

In a study in nude mice, Pupa et al. showed that ectopic expression of pRb2/p130 suppresses the tumorigenicity of the SKOV3 ovarian cancer cell line overexpressing erb-2 both in vitro and in vivo (49). No alterations

of the RB2/p130 gene were found in 43 tumors analyzed by Alvi et al. (50). In a recent study on 45 primary ovarian carcinomas, pRb2/p130 protein was lost or decreased in 40% of the specimens and the enhanced pRb2/p130 synthesis, adenovirus-mediated, leads to a drastic growth arrest in the G₁ phase of the cell cycle in ovarian cancer cell lines, suggesting its tumor suppressor function in ovarian cancer (51).

p21^{CIP1/WAF1} gene

p21 is a cyclin-dependent kinase inhibitor whose expression is usually induced by p53 and which is responsible for the p53-dependent G₁ arrest in response to DNA damage.

Barboule et al. showed that p21 is able to inhibit CDK2-kinase activity and is therefore functional in the IGROV1 ovarian carcinoma cell line. This CDK inhibitory activity is bypassed at least by overexpression of CDK2 and cyclin A, and perhaps also by PCNA overexpression (52). Among 106 patients with epithelial ovarian cancer, 61% showed p21 expression associated with early tumor stage and no residual disease after primary resection. Unexpectedly, no association with tumor grade was found. High p21 expression, which was observed in only 11% of all cases, was related to a good prognosis. The clinical follow-up showed a better overall survival for cases with strong p21 expression versus cases with weak expression or no expression ($P=0.033$) (53). The association of p21 status with clinicopathological parameters and clinical outcome was also investigated in a series of 102 ovarian tissue samples including normal ovary, primary ovarian tumors, omental metastasis, recurrent disease and residual tumor after chemotherapy exposure. In the group of stage III-IV ovarian cancer patients, p21-positive cases showed a more favorable prognosis with respect to p21-negative cases: the 3-year time to progression (TTP) rate was 58% for p21-positive cases compared to 33% of p21-negative cases ($p=0.036$) (54).

The expression of p21 was also assessed by immunohistochemistry in epithelial ovarian malignancies in relation to p53 status, cell proliferation and patient survival. Low p21 expression was significantly associated with high-grade tumor ($P=0.0005$), advanced FIGO stage ($P=0.001$) and primary residual tumor ($P=0.0001$). Low levels of p21 were also considered a marker of poor overall survival. The combination of p53 expression with the absence of p21 expression was strongly associated with poorer disease-free and overall survival, and p21/p53 expression independently predicted tumor recurrence (55,56). Conversely, the combination of p21-positive and p53-negative cases was found to be a better independent indicator of prognosis and survival in patients with ovarian carcinoma than either p21 or p53 alone (57). In a recent study on a series of 267 patients, Rose et al. surprisingly found, for the first time, compromised survival for patients with p53-null/p21-positive tumors ($P=0.005$) (58). No p21 methylation was observed in a recent study (59).

p21 was found to be overexpressed in 48% of a series of 185 uniformly treated patients with stage III ovarian cancer but did not show prognostic significance. p21 was not found to be predictive for response to chemotherapy in this large group of patients with advanced ovarian cancer (60). p21 positivity was not a significant predictor of favorable outcome. There was no relationship demonstrated between p21 expression and chemotherapy response in patients treated post-surgically with cisplatin or carboplatin as single agents or together with other chemotherapeutics (61).

By examining the effect of p21 on the response to cisplatin, Lincet et al. found that the cytotoxic effect of the drug was enhanced, as demonstrated by the increased rate of cell death (62).

p27^{Kip1} gene

The p27 gene is rarely affected by structural alterations in human

malignancies. p27 is a CKI that regulates progression from G₁ into S phase by inhibiting a variety of cyclin-CDK complexes, including cyclin D-CDK4, cyclin E-CDK2 and cyclin A-CDK2. Newcomb et al. showed that p27 expression is positively associated with long-term survival in 66% of patients separated into two equal groups, one of long-term survivors (>5 years) and the other of short-term survivors (< 2 years) (63). Baekelandt et al., in contrast with the results of Newcomb et al., demonstrated only a trend toward reduced survival (P=0.092) in 185 unselected patients. The complete loss of p27 protein expression was a rare event (6% of cases) (60).

Loss of p27 expression (33% of cases) did not correlate with any of the clinicopathological parameters used to predict clinical outcome but it was associated with short time to progression of the disease in 82 ovarian cancer patients analyzed by Masciullo et al. This association was also retained after the exclusion of stage I and stage II tumors, further supporting the hypothesis that loss of p27 confers a more aggressive phenotype to tumor cells and, therefore, might play an important role in the development of ovarian cancer (64).

Masciullo et al. also demonstrated that expression of p27 is a strong predictor of longer time to progression and overall survival in 99 patients with advanced stage ovarian cancer. Moreover, the association between loss of p27 expression and poor survival remained significant after stratification according to the residual tumor at the first surgery, which represents a parameter that plays a major role in affecting response to chemotherapy and survival.

p27-positive cases showed a higher percentage of response to chemotherapy especially in the group of patients optimally cytoreduced at the first surgery (65).

In another study, p27 expression was detected by immunohistochemistry in 75.8%, 78.3% and 36.2% of cells in benign, borderline and malignant tumors, respectively. Western blot analysis also showed lower expression of p27 in ovarian carcinomas than in benign tumors. In addition, loss of p27 expression was associated with tumor grade (P=0.003), lymph node metastasis (P=0.002) and residual disease (P=0.016) (42). The discrepancy with the results obtained by Masciullo et al. may be explained by different criteria for interpretation of the immunohistochemical pattern of p27 expression as well as for patient selection.

Sui et al. examined Jab1, a transcriptional coactivator of AP1 proteins (especially c-Jun and Jun D), and p27 protein expression in 80 ovarian carcinomas. Jab1 expression increased from normal ovarian epithelium to benign tumor, to malignant tumor. An inverse statistically significant correlation between Jab1 and p27 expression was found in both benign (P=0.003) and malignant (P=0.002) ovarian tumors. The authors suggested that Jab1 can specifically interact with p27 protein and can accelerate its degradation (66). p27 degradation is thought to be the main mechanism responsible for the downregulation of p27 protein in human tumors (67) since the transcriptional mechanism of this gene is not altered. This suggests that proteins involved in p27 degradation may have oncogenic properties such as Skp2, a protein known to be a component of a ubiquitin ligase complex specific for p27. In fact increased Skp2 levels are associated with reduced p27 expression, suggesting that increased Skp2 expression may have a causative role in decreasing p27 expression in epithelial ovarian tumors (68). p27 protein modulation is likely to be also involved in all-trans retinoic acid (ATRA)-induced growth inhibition in ovarian carcinoma cells, given that this protein was found to be upregulated in these cells following ATRA treatment (69).

In a recent study, Plisiecka-Halasa et al. performed immunohistochemical analysis of multiple biomarkers in 204 ovarian cancer patients. They demonstrated that overall survival was positively influenced by p21 plus p27 expression only in p53-negative patients. They concluded

that, considering multiple parameters, the prognostic value of p27 was strongly determined by p53 status (70).

p53 gene

As p53 has been widely reviewed in the literature, here we will briefly point out its role in ovarian cancer. It is well known that somatic mutation of p53 represents the most common molecular genetic alteration occurring in epithelial ovarian carcinoma. Inactivation of p53 was detected in 30-80% of ovarian carcinoma (71,72). The frequency of p53 mutation in early-stage ovarian carcinomas of serous histology is comparable to that reported for advanced-stage tumors, and it is therefore likely to occur early in the progression of the most common histological variant of ovarian carcinoma (73). For invasive carcinomas, the rate of mutation and expression increases with increasing tumor grade and stage, and is more common in tumors of serous histology (74). In addition to germline BRCA1 and BRCA2 mutations, somatic p53 alterations leading to p53 accumulation is an important event in hereditary ovarian cancer and is as frequent as in non-BRCA-related ovarian cancer (75). Epithelial ovarian tumors showing p53 alterations are significantly less sensitive to chemotherapy and more aggressive than those with functional p53 and overall survival is shortened in patients with p53 mutations (76,77).

CONCLUSIONS

Among the G₁ regulators, cyclin D1, CDK4, and p16 play a crucial role in ovarian cancer tumorigenesis and development. Cyclin D1 seems to be the most involved cyclin in ovarian tumors among the D-type cyclins. Low expression of cyclin D1 seems to promote the development of ovarian tumors. It is overexpressed in borderline and invasive tumors although there is no association with clinical outcome. Cyclin D2 overexpression is characteristic of a single histological tumor type, the granulosa cell tumor, which accounts for 6% of all ovarian malignancies. CDK4 activity increases in malignant tumors with respect to benign tumors, suggesting its important role in ovarian carcinogenesis. The tumor suppressor p16 (INK4a gene), like p15 (INK4b gene), when deleted is an indicator of poor chemotherapy response and unfavorable prognosis. There are controversial results about the methylation status of the p16 promoter. No evidence of hypermethylation was found in a high percentage of p16-negative cases in a number of different studies; however, there are some reports demonstrating hypermethylation of p16 promoter in a significant number of negative cases. Also, the prognostic value of the status of p16 in ovarian carcinomas is controversial. There are some data demonstrating the association between p16 positivity and poor prognosis as well as data demonstrating the lack of correlation between p16 expression and overall survival. Lack of p16 is associated with p53 wt and is typical of mucinous and endometrioid tumors. Ovarian tumors that express both p16 and RB are insensitive to p16 introduction in the ovarian cancer cells, suggesting that tumors expressing both genes may be unresponsive to p16 gene therapy. Little is known about the role of p15 in ovarian carcinomas. No mutations have been detected. Its inactivation may be the histological type-specific event in ovarian tumorigenesis. Alterations in p15 gene occur in serous, endometrioid and clear cell carcinomas but not in mucinous carcinomas. Most of the G₁-S regulators play an important role in ovarian cancer due to the fact that they control the G₁-S transition, a crucial step in cell cycle control. Cyclin E is a key regulator of the G₁-S transition. Abnormalities in cyclin E expression have been related to survival in a variety of cancers. In ovarian cancer, cyclin E overexpression is a frequent event and is closely associated with the malignant biological feature of ovarian tumors as suggested by the observation that its expression increases from benign

to borderline to malignant tumors and correlates with advanced clinical stage and poor survival. CDK2, like CDK4, is also associated with the malignancy of ovarian tumors. The member of the CIP/KIP family p27 plays an important role in ovarian cancer development, as suggested by the observation that its loss confers a more aggressive phenotype to tumor cells; in fact, its expression decreases from benign to borderline to malignant tumors. p27 expression is positively associated with long-term survival. The prognostic value of p27 is strongly determined by p53 status. A better overall survival is linked to p53 status (p53-negative) in the presence of p21 expression. The role of p21 as a prognostic factor alone or in association with other markers has been widely investigated. Low p21 expression can be considered a marker of poor overall survival. Conversely, patients with strong p21 expression versus those with weak or no expression show a better overall survival. p21 does not seem to be predictive for response to chemotherapy. Compromised survival is associated with p53-null/p21-positive tumors, but this combination is a better independent indicator of prognosis and survival than either p21 or p53 alone. The actual mutation of p53 is the most common molecular alteration occurring in both early-stage and invasive ovarian carcinomas, especially in those of serous histology, and confers resistance to chemotherapy and shortened overall survival. The retinoblastoma family member RB seems to have a role in the carcinogenesis of some human ovarian tumors. Loss of pRb expression might contribute to enhanced proliferation in early ovarian tumorigenesis, but in later stages, the carcinomas might become independent of pRb expression. Since RB and p16 tumor suppressor genes function in the same pathway of cell cycle control, most advanced tumors show a direct correlation between pRb and p16. Diminished pRb levels are related to several clinicopathological indicators of aggressiveness in ovarian adenocarcinomas such as increasing grade, advanced stage and bulk residual disease. There is direct genetic evidence that defects in p53- and pRb-mediated pathways cooperate in ovarian carcinogenesis. pRb2/p130 is down-regulated in 40% of ovarian carcinomas and its tumor suppressor function has been demonstrated in ovarian cancer cells but no mutations of this gene have been detected in ovarian tumors.

Figure 2) Deregulation of cell cycle machinery in ovarian carcinomas. Downregulated genes are indicated in yellow, whereas upregulated ones are shown in red. CDK4 and cyclin D overexpression has been reported to be associated with low p16 expression (see arrow with double heads). The upregulation of cyclin D and the downregulation of p16 leads to enhanced pRb phosphorylation, thus inducing its inactivation. p27 downregulation leads to an increase in cyclinE-CDK2 activity that contributes to ovarian carcinoma development. p15 is frequently affected by homozygous deletion in ovarian carcinoma (p15 deletion is indicated by a diamond shape). High p21 expression is associated with higher CDK2 levels (see arrow with double heads).



The described abnormalities of cell cycle regulators in ovarian carcinomas (Figure 2) suggest that most of the cell cycle regulatory genes play a crucial role in ovarian cancer tumorigenesis and/or development. The main goal in cancer therapy remains an early diagnosis of the disease, and some of the cell cycle genes described could be useful markers for achieving this goal, and therefore more targeted therapies.

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