Case Reports

Squamous cell carcinoma arising in mature cystic teratoma of the ovary: report of two cases with molecular analysis

P. Paliogiannis[1], A. Cossu[1], G. Capobianco[2], M.C. Sini[3], G. Palomba[3], G. Virdis[2], M. Dessole[2], G. Palmieri[3]

[1]Department of Surgical, Microsurgical and Medical Sciences, University of Sassari, Sassari
[2]Department of Surgical, Microsurgical and Medical Sciences, Gynecologic, and Obstetric Clinic University of Sassari, Sassari
[3]Institute of Biomolecular Chemistry, Cancer Genetics Unit, C.N.R., Sassari (Italy)

Summary

Mature cystic teratoma (MCT) is the most frequent ovarian tumor and it is generally composed of well-differentiated elements which, nevertheless, have the potential for malignant transformation. The authors report two cases of squamous cell carcinoma (SCC) arising on ovarian MCT. In the present study, no mutation of the *CDKN2A* gene, whose impairment may deeply affect either the $p16^{CDKN2A}$-CyclinD1-pRb cascade or the $p14^{CDKN2A}$-mdm2-p53 cascade, was observed in tumour tissues from our cases' collection. This suggests that changes in the protein levels for the above-described candidate effectors may be somehow due to epigenetic alterations into the mechanisms controlling their expression. Analogously, no genetic modification among the two main genes (*EGFR* and *KRAS*) upstream the MAPK signalling pathway, which has been widely reported to play a major role in both development and progression of vast majority of malignant tumours, was detected in this series. Additional genes and pathways should be therefore investigated in order to identify genomic impairments underlying the MCT malignant transformation.

Key words: Ovary; Mature Cystic teratoma (MCT); Squamous Cell carcinoma (SCC); Double-colour FISH analysis.

Introduction

Mature cystic teratoma (MCT) is the most frequent ovarian tumor, accounting approximately for 10% to 20% of all ovarian tumors [1, 2]. It is generally composed of well-differentiated elements which, nevertheless, have the potential for malignant transformation. Such event is globally rare, occurring in 0.3% to 6.67% of MCTs [3, 4]. The most common malignant tumor arising on MCT is squamous cell carcinoma (SCC), which derives from malignant ectodermal transformation, and accounts for 70% to 85% of all the MCT malignancies [5, 6]. Numerous other less common malignant forms have been reported, including adenocarcinoma, carcinoid, carcinosarcoma, melanoma, small cell carcinoma, chondrosarcoma, and others [5, 6]. Generally, tumors arising by malignant MCT transformation have greater mean dimensions than benign MCTs, and involve younger individuals [7].

The clinical and oncological management of SCCs arising on MCT is generally challenging. This is because no evidence-based guidelines exist, given the rarity of these tumors and the small number of cases reported in literature. Furthermore, preoperative diagnosis is extremely difficult, as neither clinical nor radiological pathognomonic elements have been described. Moreover, pathological diagnosis can be demanding in some cases, despite that morphological and immunohistochemical patterns of SCCs have been extensively described [8]. In addition, the prognosis of the disease is really poor, and invasive and radical surgical operations in early stages represent the only hope for cure [9]. These characteristics make the management of patients with SCC of ovarian MCT challenging and delicate.

A consistent number of cases of SCC arising on MCT have been recently published containing a increasing amount of useful clinical and oncological data. Nevertheless, very little knowledge exists concerning the molecular and pathophysiological mechanisms of MCTs' malignant transformation. In this regard, the deregulation of the cyclin-dependent kinase inhibitor 2 (*CDKN2A*) gene seems to play a role in such a type of tumorigenesis. The *CDKN2A* gene encodes two proteins, $p16^{CDKN2A}$ and $p14^{CDKN2A}$, that act as tumor suppressors [10]. In particular, $p16^{CDKN2A}$ is part of the G1–S cell cycle checkpoint mechanism, whose final effectors are CyclinD1 and pRb proteins, whereas $p14^{CDKN2A}$ exerts its tumour suppressor effect through activation of the antiapoptotic p53 protein. Alteration of the $p16^{CDKN2A}$ and p53 expressions have been reported in MCT malignant transformation [5]. An additional pathway usually associated with the pathogenesis of carcinomas is the *mitogen-activated protein kinase* (MAPK) signal transduction cascade, whose main effectors are represented by EGFR, KRAS, and BRAF gene products [11].

Revised manuscript accepted for publication July 23, 2013

The authors report two cases of SCC arising on ovarian MCT, with the aim to describe the clinical and oncological features of these tumors and to discuss the findings of the molecular analysis performed.

Cases Report

Case one

The first patient was a 47-year-old Caucasian woman, P0000, married, without significant previous pathology or surgical history, menarche at the age of 12 years, and menstrual regularity. She underwent clinical evaluation for pain in the low abdomen quadrants in the last three months. Gynecological examination showed normal conditions of the vulva and vagina, but the uterus, despite regular in size, was posteriorly displaced by a swelling mass of hard consistency extending to the umbilical line cross.

Ultrasound examination evidenced a 117 x 123 x 90 mm left ovary-linked cystic mass with an inhomogeneous, strongly distorted echo-structure. Areas of solid appearance mixed to granular and heterogeneous hypoechoic areas were also evidenced. The bladder was displaced towards the anterior pelvic wall. Power and color-Doppler ultrasound showed a poorly represented vascular tree with high strength velocimetric flow waves. The uterus, located caudally and in slight anti-flexion, presented dimensions a little higher than normal values and a globular shape with strongly inhomogeneous echo-structure due to fibromatosis. The endometrium appeared proliferative and 7.2 mm thick. No alterations of the right ovary were detected. Magnetic resonance imaging (MRI) confirmed these findings. Serum Ca19-9 was 341.37 > U / ml [normal range: 0.00-37.00], while no alterations of other tumor markers were found.

The patient underwent laparotomy and total hysterectomy, salpingo-oophorectomy, pelvic lymphadenectomy, appendectomy, and omentectomy. The postoperative course was uneventful and she was discharged at the 7th postoperative day.

Gross examination of the specimens evidenced a partially cystic mature teratoma of the ovary containing hair, sebaceous yellowish material, and a solid component. The wall of the cyst showed keratinized stratified squamous epithelium, hair follicles, sebaceous glands, and a vast foreign-body giant cell reaction. Furthermore, a vegetant SCC with wide necrotic areas and marked cytological atypias, infiltrating the wall of the dermoid cyst (but not the capsule), was also evidenced (Figure 1). Neoplastic endovascular emboli were found; no lymph node or omental metastasis were detected.

The patient underwent subsequently six cycles of platinum-based adjuvant chemotherapy and taxol and she is alive and disease-free 48 months after diagnosis. Currently the woman is free from disease.

Case two

The patient of the second case was a 57-year-old Caucasian woman, married, in P4004 (four spontaneous deliveries), menarche at the age of 13 years, and menstrual regularity. She underwent operative hysteroscopy and excision of a submucosal myoma three years prior. She was referred for gynaecological evaluation due to the presence of pain in the right iliac fossa for more than a year.

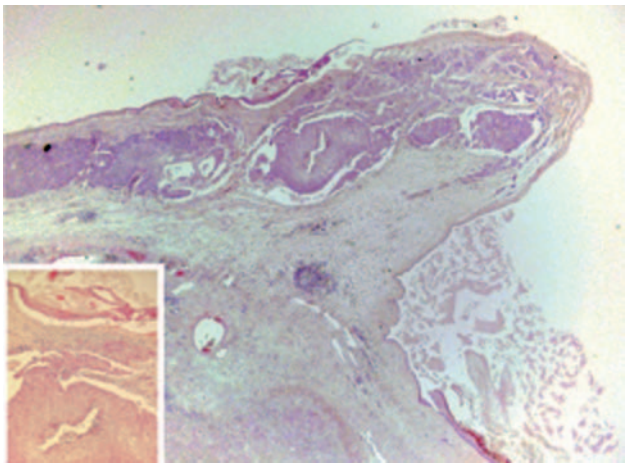

Figure 1. — Histological section showing a cyst lined by a squamous epithelium with a contigous area of squamous carcinoma (H&E x30). The malignant component is more appreciable in the inset (left lower corner)

Clinical examination of the pelvis evidenced a swelling mass of about ten cm, that was extended to the right iliac fossa. Increased levels of serum CA 19-9: 148 IU / ml and CA 125 were found.

A computed tomography (CT) scan of the abdomen and pelvis was performed showing a voluminous cystic lesion of about ten cm in greatest diameter in relation to the right adnexa. The lesion had regular margins and a partially cystic appearance with areas of fluid or fatty tissue. There were not substantial densitometric modifications after contrast administration, unless a modest parietal enhancement. There were not relevant pathologic findings in pelvic and abdominal viscera or lymph nodes. The bladder appeared imprinted by the mass on the right antero-lateral wall. An MRI of the pelvis was also performed, showing the mass of the right adnexa, which appeared hyper-intense on long TR images and hypo-intense on T1, but containing a hypo-intense on T2 and heterogeneously hyper-intense on T1 area (fat tissue). These features appeared suspicious for a MCT with a mixed fluid and fatty tissue component.

The patient underwent adhesiolysis, total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy. She was discharged at the 8th postoperative day, as no complications were observed during hospital stay.

Gross and histological examination of the specimens evidenced findings similar to those of the first case. In the context of the wall of the cystic part of the lesion, proliferation of keratinized epithelial elements with numerous atypical mitoses and areas of necrosis was evidenced (Figure 2). Some neoplastic emboli were also found, but nor infiltration of the capsule neither lymph node metastasis were detected.

The patient underwent the same chemotherapy as the previous case, but she died 30 months after surgery because of liver metastasis.

Molecular analysis

Paraffin-embedded tumour tissues from the two cases were used for both isolating genomic DNA for mutation analysis and preparing tissue sections for fluorescence *in situ* hybridization (FISH)

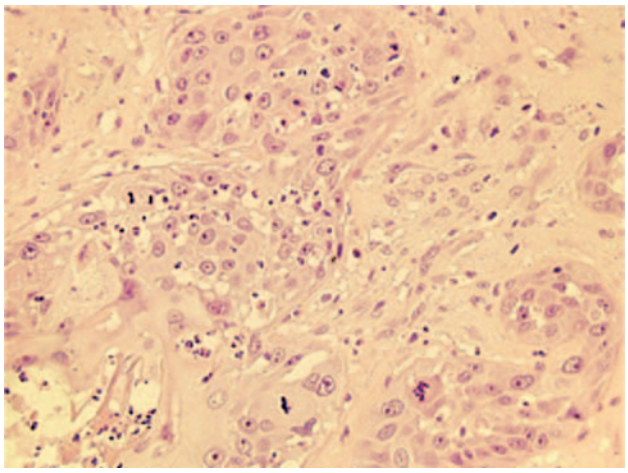

Figure 2. — Malignant squamous cells with marked anaplasia and atypical mitoses (H&E x400).

analysis. For mutation analysis, the full coding sequences and splice junctions of *CDKN2A* (exons 1, 2, and 3), *EGFR* (codons 19-21), and *KRAS* (exons 2-3) were screened for mutations through direct sequencing using an automated fluorescence- cycle sequencer. Double-colour FISH analysis was performed using probes specific for *CyclinD1* and *EGFR* genes, as previously described [12]. Mutation analysis and FISH were performed for either the malignant and the benign squamous component of MCT in both cases, in order to evidence different molecular alterations. No mutation or genomic rearrangement in candidate genes was detected in this series, with the exception of a polymorphism in exon 3 of *CDKN2A* (C500G) for the second case only.

Discussion

MCT is the most common ovarian tumor comprising ten to 20% of all tumors of the ovary, and it is bilateral in nine to 16% of cases [1, 2, 13]. Despite it is composed by well-differentiated cells, malignant transformation occurs in 0.3% to 6.67% of cases and can arise in any of the three germ layers [3, 4]. The ectoderm is most commonly involved in malignant transformation; therefore SCC is the most frequent malignancy, accounting for 70% to 85% of cases [5, 6]. Adenocarcinoma accounts for approximately seven to 15% of cases, while numerous other less common malignancies have been described [5, 6, 13]. Sporadic cases of multiple synchronous tumors arising in dermoid cysts have also been described [10].

MCT-related SCC arises frequently in post-menopausal women, as opposed to MCT which generally affects younger women. In the study of Futagami *et al.* the mean age of patients with MCT-related SCC was 42.5 years, and that of patients with benign MCT 34.2 years, while in the study of Kikkawa *et al.* the respective figures were 55.2 and 37.5 years [7, 14]. Hachethal *et al.* in a review of 277 published cases found that the mean age of patients with SCC malignant transformation was 55 years [9]. It was speculated that malignant transformation occurs in long-standing benign MCTs and this explains the tendency to affect older women. This tendency was confirmed also in the present cases.

The clinical presentation is generally similar to that of benign MCTs, characterized by abdominal pain and a mass in the pelvis, as observed in the present cases. Furthermore, signs and symptoms of compression or invasion of other anatomical structures, like bowel, ureters and bladder may be found [8, 9]. Compressive symptoms may be more frequent in cases of malignant transformation as these lesions are generally more voluminous than classic MCTs. Weight loss or fever can also be observed. These clinical manifestations may pose the suspect of a MCT, which is relatively frequent, but do not give any information on the existence of malignant transformation.

The role of several tumoral markers in the preoperative assessment of the disease has been investigated. In both the present cases, serum CA 19-9 was found increased, and CA 125 and CEA were found within normal ranges. Other authors have reported CA 19-9 increment in SCC malignant transformation with extremely variable percentages [6, 9]. More interesting appears the role of SCC antigen, despite it cannot be used for early diagnosis as it depends on the volume of the tumor [4]. It has been speculated that the combination of increased serum SCC antigen levels (> 2.5 ng/ml) and age > 40 years suitably predict malignant transformation in MCT [15]. In the study of Hackethal *et al.*, serum SCC antigen was increased in 86.5% of the cases examined; the corresponding figures for CA 125, CA 19-9 and CEA were 71%, 77% and 67%, respectively [9]. The authors did not evidence any correlation between the serum levels of these markers and the FIGO stage of the disease. Conversely, elevated SCC antigen and CA 125 were associated to poor prognosis [9]. Other markers, like TPA and M-CSF, have been reported to predict malignant transformation, but their clinical role is not clear [16].

Concerning imaging, CT and MRI represent the radiological tools most frequently used. These techniques permit generally to diagnose MCTs preoperatively. Nevertheless, differential diagnosis between a benign and malignant MCT is extremely challenging. CT and MRI can provide useful information on the extension of the disease, but their precise impact on the management of patients with MCT-related SCC must be better assessed [17].

On gross examination, MCTs generally present a mixed composition, with variable solid and cystic components and often filled with pultaceous material, hair, cartilage, bone, and other differently differentiated tissues. The surface can be smooth and regular or distorted by tumoral invasion and adhesions. Events like malignant transformation, haemorrhage, and necrosis involve mainly the solid component. The former must be carefully ruled out, because of its influence on prognosis. The diagnosis of squamous malignant transformation is based on the identification of architectural and

cytological morphologic patterns witch resemble those of the normal squamous mucosae. Well-differentiated squamous elements may present keratinization and intracellular bridging, as well as a polygonal shape and central nuclei with one or two central nucleoli. These features may be partially identifiable in less differentiated forms; immunohistochemistry becomes mandatory to reach diagnosis in these cases, as well as in cases with confusing elements like papillations, cysts, pseudoglands, and polypoid or insular patterns [8]. Typical immunohistochemical findings in SCCs are high-molecular-weight cytocheratin positivity (e.g. cytocheratins 5/6 and 34BE12) and p63 positivity [8]. In the study of Iwasa *et al.* 21 cases of MCT-related SCC were analysed and immunoreactivity for p53, MDM2, p21, and CyclinD1 was evidenced in 67%, 43%, 14%, and 57%, respectively. The same authors report decreased expression of p16 and Rb protein in 86% and 48% of cases, respectively [5]. In the present study, no mutation of the *CDKN2A* gene, whose impairment may deeply affect either the $p16^{CDKN2A}$-CyclinD1-pRb cascade or the $p14^{CDKN2A}$-mdm2-p53 cascade, was observed in tumour tissues from the cases' collection. This suggests that changes in the protein levels for the above-described candidate effectors may be somehow due to epigenetic alterations into the mechanisms controlling their expression. Analogously, no genetic modification among the two main genes (*EGFR* and *KRAS*) upstream the MAPK signalling pathway, which has been widely reported to play a major role in both development and progression of vast majority of malignant tumours, was detected in the present series. Additional genes and pathways should be therefore investigated in order to identify genomic impairments underlying the MCT malignant transformation.

Surgery represents the most important treatment method for SCC arising on MCT. Unfortunately, the diagnosis of the disease is commonly made by postoperative pathological examination of the surgical specimen, because the clinical and radiological elements mentioned above do not allow pre-operative diagnosis. This has a negative impact on the surgical planning. Nevertheless, encouraging results have been described, especially in early stages. Abdominal hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and lymphadenectomy has been proposed as the surgical procedure of choice, but in younger patients who desire to conserve the possibility to procreate and with Stage Ia disease, a monolateral approach seems to offer good oncological results [18]. The rationale of the surgical treatment of MCT-related SCC depends on the necessity for an appropriate staging of the disease and for an optimal debulking. Several authors reported that cytoreduction was one of the factors that impacts positively on survival, especially when followed by adjuvant therapy [18, 19]. This was also the rationale of the surgical strategy in both the present cases, but it was demonstrated effective only in one case.

Concerning adjuvant treatments, several chemotherapic and/or radiotherapic approaches have been proposed, but the low incidence of the disease renders evaluation and standardization of these treatments difficult. The most effective and diffusely used agent seems to be cisplatin [18]. Both the present patients underwent cisplatin-based adjuvant chemotherapy, which resulted effective, in association with previous surgery, in one case. Our oncologists and radiotherapists did not prescribe radiation therapy, as no convincing evidence exists on its usefulness [18, 19]. Some novel combined adjuvant approaches with encouraging results have been recently proposed; the most important seem to be cisplatinum-taxane chemotherapy and chemoradiation with nedaplatin [18, 20].

Several factors have been described to influence prognosis of MCT-related SCC; the most relevant seem to be age, tumor markers (SCC antigen and CA 125), tumor size, clinical stage, grade of differentiation, capsular, and/or vascular invasion and cytoreduction [9, 21]. In patients with favourable conditions and a localized, well-differentiated and well capsulate lesion, who underwent successful surgical resection prognosis is globally good, as observed in the first patient. In cases of extra-capsular invasion, prognosis is generally poor, despite surgical and chemotherapic efforts. This picture reflects the absolute need to improve and to invent diagnostic means for early detection of these tumors, which seems to be the only way to reduce mortality.

On the other hand, it is very important to better understanding the pathophysiology and molecular mechanisms of squamous cell malignant transformation, in order to enlarge the actual therapeutic possibilities. Some authors believe that the transformation process occurs through dysplastic changes in the squamous epithelium or in the columnar epithelium that had undergone squamous metaplasia [21]. Complex chromosome aberrations, alterations in the p53 and p16 genes, and in expression of cyclooxygenase-2 have been reported to play substantial roles in this setting, but the causes and the exact molecular mechanisms are not known [5, 22].

References

[1] Futagami M., Yokoyama Y., Mizukami H., Shigeto T., Mizunuma H.: "Can malignant transformation in mature cystic teratoma be preoperatively predicted?". *Eur. J. Gynaecol. Oncol.*, 2012, *33*, 662.

[2] Ulker V., Numanoglu C., Akbayir O., Akyol A., Tuncel A., Akca A., Aydin O.: "Malignant transformation arising from mature cystic teratoma of the ovary: a report of six cases". *J. Obstet. Gynaecol. Res.*, 2012, *38*, 849.

[3] Peterson W.F.: "Malignant degeneration of benign cystic teratomas of the overy; a collective review of the literature". *Obstet. Gynecol. Surv.*, 1957, *12*, 793.

[4] Bal A., Mohan H., Singh S.B., Sehgal A.: "Malignant transformation in mature cystic teratoma of the ovary: report of five cases and review of the literature". *Arch. Gynecol. Obstet.*, 2007, *275*, 179.

[5] Iwasa A., Oda Y., Kurihara S., Ohishi Y., Yasunaga M., Nishimura I., Takagi E., Kobayashi H., Wake N., Tsuneyoshi M.: "Malignant transformation of mature cystic teratoma to squamous cell carcinoma involves altered expression of p53- and p16/Rb-dependent cell cycle regulator proteins". *Pathol. Int.*, 2008, *58*, 757.

[6] Filippakis G.M., Lagoudianakis E.E., Genetzakis M., Antonakis P., Papadima A., Boussiotou A., Katergiannakis V., Manouras A.: "Squamous cell carcinoma arising in a mature cystic teratoma of the ovary with synchronous invasive lobular breast cancer: case report". *Eur. J. Gynaecol. Oncol.*, 2006, *27*, 537.

[7] Kikkawa F., Nawa A., Tamakoshi K., Ishikawa H., Kuzuya K., Suganuma N., Hattori S., Furui K., Kawai M., Arii Y.: "Diagnosis of squamous cell carcinoma arising from mature cystic teratoma of the ovary". *Cancer*, 1998, *82*, 2249.

[8] Mahe E., Sur M.: "Squamous lesions of the ovary". *Arch. Pathol. Lab. Med.*, 2011, *135*, 1611.

[9] Hackethal A., Brueggmann D., Bohlmann M.K., Franke F.E., Tinneberg H.R., Münstedt K.: "Squamous-cell carcinoma in mature cystic teratoma of the ovary: systematic review and analysis of published data". *Lancet Oncol.*, 2008, *9*, 1173.

[10] Pomerantz J., Schreiber-Agus N., Lie´ geois N.J.: "The Ink4a tumor suppressor gene product, 19Arf, interacts with MDM2 and neutralizes DM2's inhibition of p53". *Cell,* 1998, *92*, 713.

[11] Vogelstein B., Kinzler K.W: "Cancer genes and the pathways they control". *Nat. Med.*, 2004, *10*, 789.

[12] Sini M.C., Manca A., Cossu A., Budroni M., Botti G., Ascierto P.A., Cremona F., Muggiano A., D'Atri S., Casula M., Baldinu P., Palomba G., Lissia A., Tanda F., Palmieri G.: "Molecular alterations at chromosome 9p21 in melanocytic nevi and melanoma". *Br. J. Dermatol.*, 2008, *158*, 243.

[13] Savitchi E., Rao S.: "Squamous cell carcinoma and pleomorphic sarcoma (MFH) arising in a mature cystic teratoma of the ovary". *Int. J. Gynecol. Pathol.*, 2012, *31*, 443.

[14] Futagami M., Yokoyama Y., Mizukami H., Shigeto T., Mizunuma H.: "Can malignant transformation in mature cystic teratoma be preoperatively predicted?". *Eur. J. Gynaecol. Oncol.*, 2012, *33*, 662.

[15] Mori Y., Nishii H., Takabe K., Shinozaki H., Matsumoto N., Suzuki K., Tanabe H., Watanabe A., Ochiai K., Tanaka T.: "Preoperative diagnosis of malignant transformation arising from mature cystic teratoma of the ovary". *Gynecol. Oncol.*, 2003, *90*, 338.

[16] Suzuki M., Kobayashi H., Ohwada M., Terao T., Sato I.: "Macrophage colony-stimulating factor as a marker for malignant germ cell tumors of the ovary". *Gynecol. Oncol.*, 1998, *68*, 35.

[17] Park S.B., Kim J.K., Kim K.R., Cho K.S.: "Preoperative diagnosis of mature cystic teratoma with malignant transformation: analysis of imaging findings and clinical and laboratory data". *Arch. Gynecol. Obstet.*, 2007, *275*, 25.

[18] Sakuma M., Otsuki T., Yoshinaga K., Utsunomiya H., Nagase S., Takano T., Niikura H., Ito K., Otomo K., Tase T., Watanabe Y., Yaegashi N.: "Malignant transformation arising from mature cystic teratoma of the ovary: a retrospective study of 20 cases". *Int. J. Gynecol. Cancer*, 2010, *20*, 766.

[19] Chen R.J., Chen K.Y., Chang T.C., Sheu B.C., Chow S.N., Huang S.C.: "Prognosis and treatment of squamous cell carcinoma from a mature cystic teratoma of the ovary". *J. Formos Med. Assoc.*, 2008, *107*, 857.

[20] Ohtani K., Sakamoto H., Masaoka N., Shimada K., Kanaeda T., Kurihara M., Nagai N., Satoh K.: "A case of rapidly growing ovarian squamous cell carcinoma successfully controlled by weekly paclitaxel-carboplatin administration". *Gynecol. Oncol.*, 2000, *79*, 515.

[21] Chew I., Post M.D., Carinelli S.G., Campbell S., Di Y., Soslow R.A., Oliva E.: "p16 expression in squamous and trophoblastic lesions of the upper female genital tract". *Int. J. Gynecol. Pathol.*, 2010, *29*, 513.

[22] Yoshioka T., Tanaka T.: "Immunohistochemical and molecular studies on malignant transformation in mature cystic teratoma of the ovary". *J. Obstet. Gynaecol. Res.,* 1998, *24*, 83.

Address reprint request to:
G. CAPOBIANCO, M.D., PhD
Gynecologic and Obstetric Clinic Department
of Surgical, Microsurgical and Medical Sciences
University of Sassari
Viale San Pietro 12, 07100 Sassari (Italy)
e-mail: capobia@uniss.it