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Case Report

POLE exonuclease domain mutations in endometrial carcinoma: a case report

Maria Paola Pasciuto[1,2], Lara Felicioni[2], Claudia Zampacorta[1,2], Benedetta Ferro[1,2], Pietro Di Marino[3], Francesca Chiara Primavera[3], Alessandro Lucidi[4], Rebecca Rossetti[1], Mattia Barbareschi[5], Antonio Marchetti[1,2,6], Fiamma Buttitta[1,2,6], Emanuela D'Angelo[1,2,6]

[1] Center for Advanced Studies and Technology (CAST), University Chieti-Pescara, Italy; [2] Diagnostic Molecular Pathology, Unit of Anatomic Pathology, SS Annunziata Hospital, Chieti, Italy; [3] Clinical Oncology Unit, SS Annunziata Hospital, Chieti, Italy; [4] Department of Obstetrics and Gynecology University of Chieti, Italy; [5] S. Chiara Hospital, University of Trento, Italy; [6] Department of Medical, Oral, and Biotechnological Sciences University "G. D'Annunzio" of Chieti-Pescara

Summary
Endometrial carcinoma (EC) harboring POLE exonuclease domain mutations occurs in 5-15% of ECs and frequently affects young women with low body mass index (BMI). It presents at early stage as high grade endometrioid histotype with intense tumor infiltrating lymphocytes and has good clinical outcomes and favorable prognosis. In this article we report the case of a 32-year-old woman with endometriod EC (EEC) exhibiting a "ultramutated" molecular profile and an excellent prognosis despite tumor size and grading. Herein, to highlight the importance of defining POLE status in ECs for both clinical and therapeutic implications for patients.

Key words: endometrial carcinoma, POLE, ultramutated phenotype

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Correspondence
Emanuela D'Angelo
Department of Medical, Oral, and Biotechnological Sciences University "G. D'Annunzio" of Chieti-Pescara
E-mail: emanuela.dangelo@unich.it

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Introduction

Endometrial carcinoma (EC) is the fourth most common tumor affecting women and represents the second most common gynecologic malignancy [1,2].
The historical ECs pathogenetic classification has been improved by molecular classification, in subgroups described by The Cancer Genome Atlas (TCGA) Research Network [3]. In 2015, Talhouk et al., proposed for the first time a clinically applicable molecular-based classification for endometrial cancers [1] highlighting the importance of clinical and pathological analysis for better patient management.
Among molecular subtypes, POLEmut EC, which accounts for less than 10% of all ECs, are typically indolent, presenting at low stage in younger women with lower BMI, frequently exhibiting high grade endometrioid-phenotype and intense tumor-infiltrating lymphocytes [1,2,4].

Case report

We report the case of a young woman, aged 32 years, who presented with intermenstrual abnormal uterine bleeding and an endometrial lesion was identified at colposcopy, and so biopsies were performed. Bioptic material was fixed in formalin and imbedded in paraffin for histopathological examination. We observed a solid and glandular prolif-

eration characterized by both evident architectural and cytological atypia. We diagnosed these findings as endometrial endometrioid carcinoma grade 3 FIGO (Figs. 1-2).
The patient underwent MRI that showed an abnormal dishomogeneous formation arising from endometrial cavity. The tumor was 7 cm in its maximum diameter and filled the entire uterine cavity extending to the cervix (1B FIGO radiological staging).
New biopsies were performed for immunohistochemical analysis and we detected an elevated proliferative index by Ki-67 and a heterogeneous immunoreaction for p53 (Fig. 3).
MSI status by immunohistochemical studies was analyzed and no microsatellite instability was observed.
Tumor DNA extracted from paraffin samples has been completely sequenced through Next-Generation Sequencing (NGS), showing a large number of sequence alterations (Tab. I) and an average read depth in analyzable target regions equal to 1042. Coverage data and allelic frequency showed a minimal coverage level equal to 782 and a minimal allelic frequency equal to 12%, indicating an optimal sequencing performance.
The major pathogenetic mutations reported on database and on prediction sites were referred to POLE (Pro286Arg and Asn1396Ser), MSH6, BRCA1 and PTEN (Tab. II).

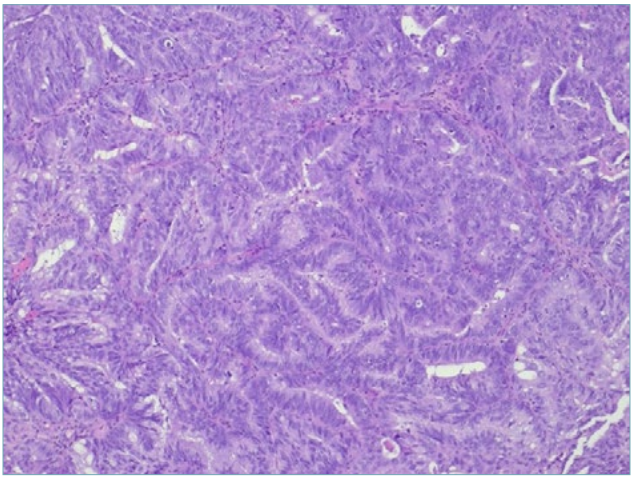

Figure 1. Endometrioid endometrial carcinoma with prominent architectural atypia (no solid pattern shown) stained in hematoxylin-eosin (10x).

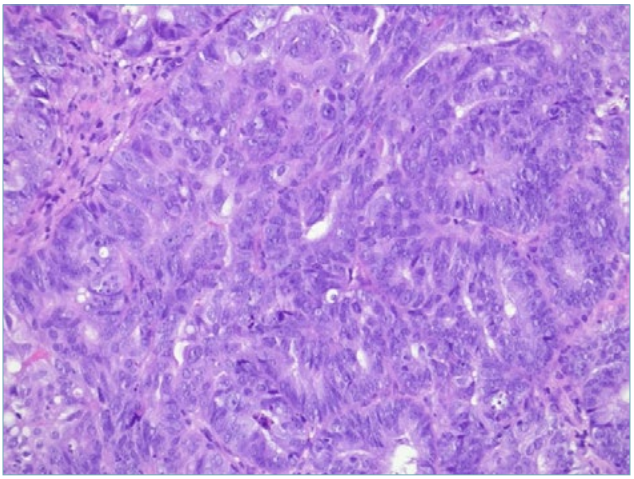

Figure 2. Endometrioid endometrial carcinoma with evident cytologic atypia (round to oval nuclei, coarse chromatin, prominent nucleoli) stained in hematoxylin-eosin (40x).

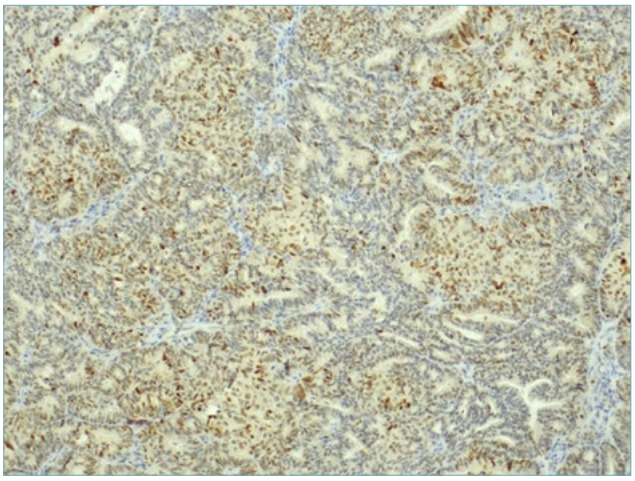

Figure 3. Endometrioid endometrial carcinoma exhibiting heterogeneous p53 expression (10x).

Discussion

Endometrial carcinoma is the most frequent tumor affecting women in the developed countries and represents the second most common malignant tumor in female patients [1,2].
Since TCGA proposed the current subgroup classification, the integration of clinical, morphological and molecular data has been highlighted by many authors, focusing attention on MSI, TP53 and POLE status and recognizing four molecular subgroups with specific therapies and prognosis [3]:

- "ultramutated" tumors harboring pathogenic POLE mutations;

Table I. Pathogenetic mutations identified in the extracted DNA from biopsies.

POLE c.857C > G p.Pro286Arg	Pathogenetic
POLE c.4187A > G p.Asn1396Ser	Pathogenetic AT 3 silico prediction sites
APC c.3058G > T p.Glu1020*	Pathogenetic
APC c.4195C > T p.Arg1399Cys	VUS
ATM c.195G > T p.Gln65His	Pathogenetic AT 4 silico prediction sites
ATM c.6848C > A p.Ser2283*	VUS
ATM c.7327C > T p.Arg2443*	Pathogenetic
BRCA1 c.2158G > T p.Glu720*	Pathogenetic
BRCA1 c.3642G > T p.Glu1038Gly	VUS
BRCA1 c.4837A > G p.Ser1613Gly	Pathogenetic AT 3 silico prediction sites
BRCA1 c.3254G > T p.Arg1085Ile	pathogenetic AT 4 silico prediction sites
BRCA1 c.3113A > G p.Glu1038Gl	Pathogenetic AT 3 silico prediction sites
BRCA1 c.407G > T p.Arg136Ile	Pathogenetic AT 3 silico prediction sites
BRIP1 c.2146A > G p.Asn716Asp	VUS
CDH1 c.1489G > A p.Glu497Lys	VUS
CHEK2 c.1654C > T p.Pro552Ser	VUS
CTNNB1 c.2013G > T p.Lys671Asn	Pathogenetic AT 3 silico prediction sites
MRE11 c.1492G > A p.Asp498Asn	VUS + 3 silico prediction sites
MSH2 c.1738G > T p.Glu580*	Pathogenetic
MSH2 c.942+2_942+9delTAAAAAAA	Pathogenetic
MSH2 c.965G > A p.Gly322Asp	Pathogenetic AT 3 silico prediction sites
MSH2 c.1166G > A p.Arg389Gln	VUS + pathogenetic AT 4 silico prediction sites
MSH6 c.1445G > A p.Arg482Gln	VUS
MUTYH c.925C > T p.Arg309Cys	VUS
NF1 c.3790G > T p.Glu1264*	Pathogenetic
NF1 c.4084C > T p.Arg1362*	Pathogenetic
NF1 c.7348C > T p.Arg2450*	Pathogenetic
PALB2 c.1061C > A p.Ser354Tyr	Pathogenetic AT 3 silico prediction sites
PTEN c.19G > T p.Glu7*	Pathogenetic
PTEN c.389G > A p.Arg130Gln	Pathogenetic
RAD50 c.2983G > T p.Glu995*	Pathogenetic

Table II. Pathogenetic mutations identified in the extracted DNA from biopsies, allelic frequency and coverage level.

Gene	DNA	Protein	Exon	AF*	Coverage	IARC**	
POLE	c.857 C > G	p.Pro286Arg	P286R	9	47%	1512	4
PTEN	c.19G > T	p.Glu7*	p.E*	1	45%	931	4
PTEN	c.389G > A	p.Arg130Gln	R130Q	5	39%	891	5
NF1	c.7348C > T	p.Arg2450*	R2450*	50	42%	1274	5
NF1	c.4084C > T	p.Arg1362*	R1362*	30	42%	1051	5
NF1	c.3790G > T	p.Glu1264*	E1264*	28	43%	1264	5
MUTYH	c.925C > T	p.Arg309Cys	R309C	10	51.5%	1354	3
MSH6	c.14445G > A	p.Arg482Gln	R482Q	4	41%	1355	3
MSH2	c.1738G > T	p.Glu580*	p.E580*	11	41%	1064	5
MRE11	c.1492G > A	p.Asp498Asn	D498N	13	42%	782	3
BRCA1	c.2158G > T	p.Glu720*	p.E720*	10	41%	1375	5
ATM	c.7327C > T	p.R2443*	p.R2443*	50	46%	930	5
APC	c.3058G > T	p.Glu1020*	p.E1020*	16	12%	934	5

*AF: allelic frequency; **IARC: International Agency for Research on Cancer

- "hypermutated" tumors, characterized by mismatch repair deficiency (MMRd);
- "NSMP" (no specific molecular profile), low copy number (p53 wild-type);
- "high copy number" (abnormal p53) [3].

In 2015, Talhouk et al. proposed, a diagnostic algo-

rithm based on a limited panel of immunohistochemistry (MMR proteins and p53 expression) and POLE mutation analyses[1].

Distinguishing the EC molecular subgroups has clinical and prognostic implications: a woman, often younger as compared to average age at diagnosis, usually presenting at early stage with high grade EC harboring pathogenic POLE mutation, benefits from conservative treatment and has an excellent prognosis [1,3,4].

POLE is the acronym for polymerase epsilon, a protein with a catalytic subunit responsible for DNA replication and repair mechanisms. POLE alterations are bounded with neoplastic transformation due to an acquired "ultramutated" phenotype (232 x10^{-6} mutations per Mb), not only in ECs but also in other malignancies, such as lung cancer and melanoma[3].

They are frequently high-grade tumors with morphological heterogeneity and/or ambiguity. At the molecular level, the majority of POLE-mutated tumors are microsatellite stable (65%), and TP53 mutations are present in 35% of cases. They also have mutations in PTEN (94%), FBXW7 (82%), ARID1A (76%), and PIK3CA (71%). Since patients with POLE-mutated tumors have an excellent prognosis, these women could benefit from conservative treatment. POLE ultramutated tumors are associated with an increase of CD8+ tumor-infiltrating lymphocytes, counterbalanced by overexpression of PDL-1 (an immune checkpoint protein) by the tumor cells, suggesting that these tumors might be excellent candidates for immune checkpoint inhibition (anti-PDL-1/PD1) [2,4,6].

In spite of the great number of TCGA-related articles that appeared over the last 10 years, utility of TCGA has been limited to the rare cases of high-grade EECs with POLE. These patients have low risk of recurrence and can benefit from a de-escalating adjuvant treatment in early stage or from immune checkpoint inhibitors. The TCGA has also highlighted the importance of requesting immunohistochemistry for mismatch repair proteins in all ECs, particularly in endometrioid carcinomas, to identify patients with Lynch syndrome and patients who may benefit from immunotherapy. Knowledge about the other three molecular groups has not changed significantly.

EEC with MMRd represents approximately 35% of EEC cases, and has similar features to POLE carcinomas, but patients have older age at diagnosis and higher BMI. EECs with MMRd have a high mutational burden (18 x10^{-6} mutations per Mb) and carry many neoantigens in the tumor cell surface. These neoantigens attract lymphocytes into the tumor, suggesting an immune-mediated role in the pathogenesis of these tumors. These tumors typically show extensive lymphocytic infiltration inside and around tumor cells. Microcystic elongated and fragmented (MELF) pattern of myometrial invasion and lymphovascular invasion are also associated with this molecular subtype.

TP53abn carcinomas account for 11% of EECs and have low mutation rate (2.7 x10^{-6} mutations per Mb). Morphologically, these are high-grade (grade 3) EECs. These tumors have poor prognosis with recurrences and peritoneal dissemination, similar to serous carcinomas. Arising in older patients with low BMI, tumors are high-grade, histologically serous, endometrioid, and carcinosarcoma, and are characterized by aggressive histopathologic parameters (diffuse LVSI, lymph node metastasis, advanced stage).

NSMP tumors represent the majority of cases, related to higher BMI, and they are predominantly early-stage low-grade endometrioid carcinomas. This molecular subgroup is genomically relatively stable, MMR-proficient (Non-specific mutation profile; NSMP) and ccounts for 39% of EECs. Tumors have moderate number of mutations (2.9 x10^{-6} mutations per Mb), mostly within the PI3K/Akt and Wnt signalling pathways. These patients have an intermediate prognosis. Recently, CTNNB1 (beta-catenin 1) and L1 cell adhesion molecule (L1CAM) mutations have been associated with poor prognosis in EEC patients with a low degree of copy-number alterations. CTNNB1 mutation has been found to be associated with more distant recurrence [REFS]. In a separate study looking at low-grade early stage endometrioid EC, CTNNB1 mutation was also shown to be associated with poorer recurrence-free survival [7,8]. L1CAM is a membrane glycoprotein that plays a role in tumor cell migration. It is a strong predictor of decreased survival and is associated with adverse clinicopathological characteristics, namely > 50% myometrial invasion, LVSI, and lymph node involvement [7,8]. L1CAM may help to classify patients otherwise unclassifiable using the traditional EC molecular classification and is strongly associated with p53 [7,8].

Conclusions

POLEmut endometrial carcinoma account for less than 10% of all ECs[2] and it is commonly associated with some of the worst prognostic factors, such as histotype (most commonly endometrioid type), high tumor grading, myometrial infiltration, lymphovascular invasion and low response to conventional treatments, as observed in preclinical data (chemotherapy and radiation), demostrating that conventional pathologic prognostic features are not so relevant in this subgroup: in fact, despite these unfavorable characteristics, POL-

Emut ECs present often at early stages (I-II) and show excellent prognosis with an indolent clinical course [2,4]. Furthermore, POLEmut ECs frequently exhibit intense tumor-infiltrating lymphocytes and/or peri-tumoral lymphocytes, as MMRd ECs [4], suggesting a potential role of inflammation in the favorable outcome and a possible target for immunotherapies and, accordingly, the Food and Drug Administration has approved the anti-PD1 (pembrolizumab) for MMRd endometrial carcinomas with recurrence [6].
Further studies are requierd to define new pathogenic POLE exonuclease domain mutations in order to assess innovative therapies especially in young patients with endometrial carcinoma.

Conflicts of interest

The authors declare no conflict of interest.

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Ethical consideration

The information contained in this manuscript complies with the journal's ethical standards.

Author contributions

All authors gave their approval for pubbliclation of the final version of the manuscript .

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