**Case report**

**Lymphoepithelioma-like carcinoma of the endometrium: Case report of a rare tumour with comprehensive immunohistochemical and molecular analysis**

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We are reporting a case of endometrial lymphoepithelioma-like carcinoma (LELC) in a 63-year-old female. Microscopically, the tumor consisted of groups of tumor cells surrounded by dense lymphoplasmacytic infiltrate. Immunohistochemically, the tumour cells were positive for cytokeratins AE1/AE3, EMA, PAX8, p16, and estrogen receptors. Protein p53 showed an aberrant type of expression. Molecular genetic analysis revealed mutations in the TP53 and PIKP53CA genes. Based on our results, we believe that the tumor represents an unusual morphological variant of endometrial serous carcinoma. To the best of our knowledge, only six cases of LELC arising in endometrium have been reported in literature to date.

**Key words:** serous carcinoma, lymphoepithelial carcinoma, lymphoepithelioma-like carcinoma, endometrium.

**Introduction**

Lymphoepithelial carcinoma (lymphoepithelioma) is an undifferentiated type of carcinoma with distinctive morphological features and common association with Epstein-Barr virus (EBV), first described in the nasopharynx [1]. This tumour is characterised by the proliferation of undifferentiated neoplastic epithelial cells, with a prominent reactive inflammatory cell infiltrate. Carcinomas with morphologically similar features occurring outside of the nasopharynx are termed “lymphoepithelioma-like carcinoma” (LELC). These tumours have been reported in various organs. The association with EBV infection in LELC is different in various locations and in different geographic regions. EBV positivity has been described in tumours of lung, stomach, thymus, and salivary gland [2, 3, 4, 5], but it has not been demonstrated in cases of LELC occurring in the urinary bladder, renal pelvis, breast, and in the female genital tract [6, 7, 8, 9, 10]. It has been suggested that EBV positivity is related to a direct organ exposure to the external environment, and EBV negativity is more common in internal organs [11]. However, in LELC of uterine cervix the association with EBV has been observed only in a few Asian patients, but not in western countries [12, 13]. In the female genital tract LELC mostly occurs in the cervix, where it is regarded as a variant of squamous cell carcinoma, but cases involving the vulva, vagina, endometrium, and ovary have also been reported [9, 10, 12, 13, 14, 15, 16, 17, 18, 19]. To the best of our knowledge, only six cases of LELC arising in the endometrium have been reported to date; however, none of these was accompanied by a molecular genetic analysis, with the exception of molecular testing for microsatellite instability (MSI) in one of the cases [14, 15, 16, 17, 18]. We report the seventh case of endometrial LELC with comprehensive immunohis-
tochemical and molecular genetic analysis [14, 15, 17, 18].

Clinical history

A 63-year-old woman presented with postmenopausal bleeding. Her gynaecological history includes menarche at the age of 14, irregular menstrual cycles, and menopause at the age of 51 years. She had had two vaginal spontaneous deliveries. Physical examination was unremarkable, and laboratory data were within normal limits. A transvaginal ultrasound showed irregular endometrial thickening, and the patient underwent endometrial curettage. Biopsy examination showed high-grade carcinoma with LELC features. Based on this diagnosis, the patient underwent radical hysterectomy with bilateral adnexectomy and pelvic and para-aortic lymphadenectomy. Biopsy examination of the resection specimen showed only residual tumour structures limited to the endometrium. The patient was staged as FIGO IA and received adjuvant vaginal brachytherapy. Six months after the surgery she is free of any signs of disease.

Material and methods

Immunohistochemistry

Sections from formalin-fixed paraffin-embedded tissue blocks were stained with haematoxylin-eosin. Selected sections were analysed immunohistochemically, using the avidin-biotin complex method with antibodies directed against the antigens listed in Table I.

In situ hybridisation (ISH)

In situ hybridisation (ISH) staining for EBV-encoded RNA (EBER) transcript was performed on deparaffinised tissue sections. The Epstein–Barr Virus (EBER) PNA Probe/Fluorescence kit was used (Dako, code Y5200) and visualised with the Dako PNA ISH Detection Kit (Dako, code K5201). For negative controls, the EBER probe was omitted.

Molecular analysis

DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tissue by cobas® DNA Sample

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK AE1/AE3</td>
<td>AE1/AE3</td>
<td>Dako, Glostrup, Denmark</td>
<td>1:50</td>
<td>Diffusely positive</td>
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<tr>
<td>EMA</td>
<td>E29</td>
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<td>1:100</td>
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<td>OR</td>
<td>6F11</td>
<td>Novocastra, Newcastle, UK</td>
<td>1:50</td>
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<tr>
<td>PR</td>
<td>16</td>
<td>Novocastra, Newcastle, UK</td>
<td>1:200</td>
<td>Negative</td>
</tr>
<tr>
<td>HNF1β</td>
<td>Polyclonal</td>
<td>Sigma-Aldrich, Prestige antibodies, St. Louis, United States</td>
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<td>Negative</td>
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<tr>
<td>Ki-67</td>
<td>MIB-1</td>
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<td>Positive in 80%</td>
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<tr>
<td>napsin A</td>
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<tr>
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<tr>
<td>synaptophysin</td>
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<td>Dako, Glostrup, Denmark</td>
<td>1:50</td>
<td>Negative</td>
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<tr>
<td>chromogranin A</td>
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<tr>
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<td>1:1600</td>
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<tr>
<td>vimentin</td>
<td>V9</td>
<td>Dako, Glostrup, Denmark</td>
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<tr>
<td>PAX 8</td>
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<td>1:50</td>
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<tr>
<td>P16</td>
<td>E6H4TM</td>
<td>Roche mtm Laboratories AG, Manheim, Germany</td>
<td>RTU</td>
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<td>MLH-1</td>
<td>G168-15</td>
<td>Spring Bioscience, Pleasanton, CA</td>
<td>1:200</td>
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</tr>
<tr>
<td>MSH-2</td>
<td>FE 11</td>
<td>Zytomed Systems, Berlin, Germany</td>
<td>1:50</td>
<td>Positive</td>
</tr>
<tr>
<td>MSH-6</td>
<td>44</td>
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<td>PMS-2</td>
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<tr>
<td>CD3</td>
<td>SP7</td>
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<tr>
<td>CD20</td>
<td>L26</td>
<td>Dako, Glostrup, Denmark</td>
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<td>Positive in B cells</td>
</tr>
</tbody>
</table>

CK – cytokeratin; OR – oestrogen receptors; PR – progesterone receptors; RTU – ready-to-use
Preparation kit (Roche; Germany). Macrodissection was performed to enrich the tumour content. Sequence capture next-generation sequencing (NGS) was performed with 300 ng of gDNA (40% of neoplastic cells). A library was constructed using KAPA HyperPlus kit, and the target area (219 kbp, 73 genes) was enriched using custom-designed hybridisation probes (Nimblegen, Roche) and pair-end sequenced using a Miseq instrument (Illumina).

Readings were aligned to the human genome (GRCh37/hg19), with an average coverage of 550.7 (median 660.3; range 2-1174). Nonsynonymous variants in exons and adjacent intronic regions with minimal coverage 100x and frequency >5% were evaluated, and manually controlled using an Integrative Genomic Viewer (Broad Institute). Copy number variations were not evaluated due to the low quality of the FFPE sample. A list of investigated genes and characteristics of detected variants is provided in Table II.

For the evaluation of the functional or clinical importance of detected missense variants widely used in silico prediction tools were utilised (part of NextGENe software: dbNSFP v 2.9 – set of algorithms for predicting the impact of the variant on protein function, according to the sequence conservation and population frequency; ClinVar; dbscSNV for evaluating the impact of splice variants). Furthermore, the IARC TP53 database (http://p53.iarc.fr) and Catalogue of somatic mutations in Cancer (COSMIC; http://cancer.sanger.ac.uk/cosmic/, accessed on October 20, 2017) were employed [20].

MSI was analysed using fragment analysis on ABI 3500, examining five quasimonomorphic mononucleotide microsatellite markers: (MSI) BAT-26, BAT-25, NR-21, NR-22, and NR-24.

Results

Material from endometrial curettage consisted of small fragments of poorly differentiated high-grade carcinoma. The tumour was composed of irregular sheets, poorly defined nests, or individual tumour cells, surrounded by dense lymphoplasmacytic infiltrate (Fig. 1). The tumour cells had indistinct margins, with round to oval vesicular nuclei with prominent nucleoli, and eosinophilic to amphophilic cytoplasm (Fig. 2). The mitotic index was up to seven mitoses per 10 high-power fields.

Immunohistochemically, the tumour cells were strongly and diffusely positive for cytokeratins AE1/AE3, EMA, PAX8, and p16 and some of them were positive for vimentin and S100 protein (Fig. 3). Protein p53 showed aberrant type of expression (positivity in more than 75% of tumour cells), which is in concordance with the detected pathogenic mutation in the TP53 gene (Fig. 4, Table II). Oestrogen receptors were positive in 75% of tumour cells. The proliferative (Ki-67) index was about 80%. Nuclear staining of the mismatch repair (MMR) proteins (MLH-1, MSH-2, MSH-6, and PMS-2) was positive, and the results of fragment analysis confirmed microsatellite stable tumour. Progesterone receptors, chromogranin A, synaptophysin, HNF1β, and napsin A were negative. EBER in situ hybridisation was negative. The lymphoid stromal infiltrate consisted of a mixture of CD20-positive B-lymphocytes and CD3-positive T-lymphocytes.

Mutation analysis was performed in 59/73 genes with sufficient coverage (14 genes in this panel were poorly covered due to the insufficient quality of DNA). Non-synonymous variants were detected in the TP53 c.584T>C (p.Ile195Thr) located in the DNA binding domain, PIK3CA genes c.323G>A (p.Arg108His) in exon 2, and c.1048G>A (p.Asp350Asn) in exon 5 (Table II). All of these variants were previously described in the COSMIC database (http://cancer.sanger.ac.uk/cosmic/) and were considered pathogenic. No patho-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Ref. Seq. Mutation</th>
<th>Predicted Effect</th>
<th>Mutant Allele Frequency %</th>
<th>Predicted Impact – In Silico/Databases and Literature</th>
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<tbody>
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<td>TP53</td>
<td>6</td>
<td>NM_001126112.2</td>
<td>p.Ile195Thr</td>
<td>18</td>
<td>Pathogenic / pathogenic</td>
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<tr>
<td></td>
<td></td>
<td>c.584T&gt;C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs760043106</td>
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<tr>
<td>PIK3CA</td>
<td>2</td>
<td>NM_006218.2</td>
<td>p.Arg108His</td>
<td>24</td>
<td>Pathogenic / pathogenic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.323G&gt;A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>c.1048G&gt;A</td>
<td>p.Asp350Asn</td>
<td>18</td>
<td>Pathogenic / pathogenic</td>
</tr>
</tbody>
</table>

*genes evaluated by NGS: AKT3, ARID1A, ARID2, ATM, BAP1, BARD1, BRAF (ex11, 15), BRCA1, BRCA2, BRIP1, CCND2, CCND3, CDH1, CDK4, CYP19A1, ERBB2, ERCC3, ESR1, ESR2, F11R, GNAQ, HNF1B, IDH1, JAM2, JAM3, KDR, KIT, KRA, MAPJ3K1, MAPK1, MDM2, MET, MITF, MLH1, MLH3, MSH2, MSH6, MYC, NBN, NTRAS, PALB2, PARD3, PDGFRα, PIK3CA, POLE, POT1, PPM1D, PPP6C, PTEN, RAD51C, RAD51D, RB1, SF3B1, SNAI2, TP53, TWIST1, ZEB1, ZEB2; AKT3, BIRC3, FOXL2, GNA11, HRAS, MAP2K2, SNAI3, TWIST2; partially evaluated because of low coverage in part of the genes: CDKN2A, MAPK3, SMARCA4, SMARCB1, SNAI1; impossible to evaluate because of low coverage: AKT1, BIRC3, FOXL2, GNA11, HRAS, MAP2K2, SNAI3, TWIST2
genic mutations were found in genes such as PTEN, ARID1A, KRAS, POLE, HNF1B, or BRCA2, which are frequently mutated in endometrial carcinomas.

Additionally, heterozygous polymorphism was detected in BRCA2, NM_00059.3: c.1114A>C (p.Asn372His, rs144848;38.2% MAF), although this finding is considered to be tolerated or neutral by in silico predictive tools.

In a subsequent hysterectomy the uterus was measured as 46 × 27 × 40 mm, with endometrial thickness of 3 mm. There were some regressive changes with only residual tumour structures in the fundus, limited to the endometrium. The cervix and both adnexa were unremarkable without any signs of tumour involvement. All 35 sampled and examined lymph nodes were without metastases.

Discussion

Endometrial carcinomas can be classified into four main histological types encompassing endometrioid, serous, mucinous, and clear cell carcinoma. These tumours can usually be distinguished based on their morphology; however, in doubtful cases ancillary methods are needed. In most cases, immunohistochemistry can be helpful, but some equivocal cases with overlapping morphological and immunohistochemical features still continue to be a diagnostic challenge. In these cases, molecular genetic analysis can be used, which allow us to classify most tumours into the correct category. Especially problematic can be the distinction between endometrioid carcinoma (EC) and endometrial serous carcinoma. Immunohistochemically, analysis of p53, p16, MMR proteins, and PTEN expression can be very helpful in this case. Endometrial carcinoma is characterised by the loss of PTEN expression, non-diffuse p16 staining, wild type p53 in most cases, and MSI. Conversely, endometrial serous carcinomas are in most cases p16 diffusely positive, show abnormal expression of p53, retained expression of PTEN, and are MSS. Regarding their genotype, endometrioid carcinomas are characterised by: 1) concurrent PTEN and ARID1A.
mutation; or ii) PTEN or ARID1A mutations in the absence of concurrent TP53 (and PPP2R1A) mutations. Serous genotype is characterised by TP53 mutation in the absence of PTEN or ARID1A mutations. PIK3CA mutation can occur in a subset of both endometrial and serous carcinomas and is not helpful in discriminating between these tumours [21].

Recent approaches classify endometrial carcinomas, based on their molecular characteristics, into four groups: POLE ultramutated (POLE), microsatellite instability-high, copy number-low (CN-L), and copy number-high (CN-H) [22, 23]. The TCGA described the differences between high-grade endometrioid carcinoma and serous carcinoma based on frequent mutations in PTEN, ARID1A, mismatch repair genes, and KRAS. While these are common in endometrioid carcinoma, serous carcinoma, however, carries frequent mutations in TP53 and PPP2R1A, and mutations in PTEN, ARID1A, mismatch repair genes, and KRAS are not frequent in this carcinoma. Generally, all serous carcinomas belong to the category CN-H, which is characterised by TP53 mutation. However, high-grade endometrioid carcinomas show substantial genetic heterogeneity and a certain proportion of them fall into the same category as serous carcinoma (CN-H tumours). Nevertheless, these cases can be distinguished by analysis of the above-mentioned genes: ARID1A and PTEN. Further studies are needed to show whether CN-H grade 3 endometrioid carcinomas are clinically different from endometrial serous carcinoma.

In our case, despite its unusual morphology, the tumour showed the typical immunophenotype (diffuse p16 staining, aberrant expression of p53) and genotype (mutation of TP53, absence of mutations of ARID1A or PTEN) of endometrial serous carcinoma. Expression of PAX8 and oestrogen receptors can be seen in both tumour types, and as such is not particularly helpful in the differential diagnosis, but it is suggestive of Müllerian origin of the tumour. Based on these features, we believe that our case represents an unusual morphological variant of endometrial serous carcinoma with LELC features due to the heavy inflammatory infiltration of tumour stroma.

Endometrial LELCs are rare tumours; only seven cases including ours have been reported to date. All of the reviewed cases were EBV negative with only one exception, and even then only a few cells showed positivity for EBV LMP1 [16]. MSI testing was performed only in one previous case (evaluated by PCR using capillary electrophoresis), and identically to our case the tumour was microsatellite stable [15]. So far, MSI was found only in a subset of gastric LELCs [24].

Regarding immunohistochemical results, all the reported endometrial LELCs showed positivity for epithelial markers in tumour cells, and CD5 and CD20 positivity in stromal cells, but other results are equivocal [14, 15, 16, 17, 18]. Positivity for oestrogen and progesterone receptors was found in one of three cases [14, 15, 17]. The aberrant type of p53 expression was found in two of the three investigated cases, in accordance with our results [14, 15]. One of the three reported cases was negative [17], but the authors did not specify whether the 'negativity' meant a complete loss of p53 expression because this finding may also, in some cases, be regarded as aberrant expression. The expression of p16 was investigated only in one previous case, with a positive result, which is also in accordance with our reported case [15]. The aberrant expression of p53 and overexpression of p16 are likely signs of manifestation of cell-cycle dysregulation, which is quite a common finding in high-grade carcinomas [15].

Differential diagnosis may include lymphoproliferative lesions/tumours, which can, however, be easily excluded by immunohistochemical recognition of their epithelial component. Moreover, large cell neuroendocrine carcinomas with LELC features were rarely described, but this entity could be ruled out based on the absence of neuroendocrine differentiation markers [25]. The most significant differential diagnostic consideration is an undifferentiated carcinoma (UC). This differential diagnosis may have certain clinical implications because some authors suggested that the prognosis of LELC is unclear or possibly favourable, whereas the prognosis of UC tends to be poor [15].

Regarding this particular differential diagnosis, the evaluation of immunohistochemical expression of epithelial markers can be helpful. LELC typically show almost diffuse positivity, in contrast to the focal or patchy expression found in UC [26].

In conclusion, endometrial LELCs are rare carcinomas that are not associated with EBV. The histogenesis of endometrial LELC is unknown; however, the immunohistochemical and molecular genetic profile in our case suggests that this tumour could represent an unusual morphological variant of endometrial serous carcinoma. Nevertheless, a comprehensive analysis of more cases is needed to fully understand the histogenesis, biologic behaviour, and prognosis of these tumours. This could, however, be problematic due to their rarity.

This work was supported by the Ministry of Health, Czech Republic (Conceplual development of research organisation 64165, General University Hospital in Prague and Project AZV 17-2840/A), by Charles University (Project Progres Q28/LF1, UNCE204065, and SVV 260367), and by OPPK (Research Laboratory of Tumor Diseases,CZ.2.16/3.1.00/24509).

The authors extend their thanks to Zachary H.K. Kendall, B.A. for English language corrections.

The authors declare no conflicts of interest.
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