CASE REPORT

Combined large cell neuroendocrine carcinoma and endometrioid carcinoma of the endometrium: a shared gene mutation signature between the two histological components

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Received: 2 June 2016 / Accepted: 10 September 2016 / Published online: 19 September 2016
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Abstract A 61-year-old Japanese woman was diagnosed with FIGO Stage IB endometrioid cancer (EC) combined with large cell neuroendocrine carcinoma (LCNEC). Metastasis to the lymph nodes in the right bronchopulmonary area, mediastinum and brain were also identified. The patient eventually developed pleuritis and pericarditis carcinomatosa, and died of cancer at 51 months after surgery. Because gene aberrations in uterine neuroendocrine carcinoma are still not well understood, we examined alterations in the mutational hotspots of 50 selected cancer-associated genes. The EC and LCNEC components shared identical alterations in *PTEN*, *PIK3CA* and *FGFR3*. Both the EC and LCNEC components had heterozygous SBSs on *CTNNB1* but at different codons (G34R in EC, and T41A in LCNEC). The altered gene signature raised a possibility that the EC and LCNEC components were derived from a common precursor lesion. The LCNEC independently obtained a significant *CTNNB1* mutation

and the lymph node metastasis originated from this component. Because the LCNEC component seemed to bring about the aggressive course of the disease and defined the patient outcome, further investigations are needed to elucidate the mechanism of NE carcinoma development in the endometrium.

Keywords Endometrial cancer · Large cell neuroendocrine carcinoma · Gene alteration · PI3 kinase activation

Introduction

Neuroendocrine (NE) carcinomas of the female genital tract are aggressive and uncommon tumors. They usually involve the cervix and ovary, and are very rarely observed in the endometrium. The overwhelming majority of endometrial NE carcinoma cases are of the conventional small cell type (up to 80 cases), and only 14 cases of large cell neuroendocrine carcinoma (LCNEC) of the endometrium have been reported in the literature. The majority of LCNEC develop in the lung. Both small cell lung cancer (SCLC) and LCNEC are considered as high-grade NE carcinoma and share several gene expression profiles [1]. However, it is controversial to consider them as a single entity when considering treatment options, and morphological and phenotypical differences between the two have been investigated [2, 3]. Here we report a case of combined LCNEC and endometrioid carcinoma (EC). To elucidate the origin of LCNEC, which determines patient outcome and death, we examined somatic gene alterations in this case. According to the mutational signatures, we discuss the origin of the LCNEC.

Electronic supplementary material The online version of this article (doi:10.1007/s13691-016-0263-9) contains supplementary material, which is available to authorized users.

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Case report

Clinical presentation

A 61-year-old Japanese woman (gravida 4, para 4) visited our hospital because of abnormal uterine bleeding for 1 year. Magnetic resonance imaging (MRI) showed a heterogeneously enhanced mass in the endometrium with suspected invasion to more than half of the myometrium on T2-weighted imaging. The patient was diagnosed preoperatively to be grade 1 (G1) EC corresponding to International Federation of Gynecology and Obstetrics (FIGO) stage IB. The patient underwent a modified radical hysterectomy with bilateral salpingo-oophorectomy, omentectomy, and pelvic lymphadenectomy. Grossly, a polypoid tumor ($3.5 \times 2.5 \times 2.1 \text{ cm}^3$) was identified in the uterine corpus.

Histologically, the majority of the tumor tissue was revealed to be G1 EC but associated with a solid area (Fig. 1a, b). The EC component accounted for 75 % of the tumor, while the solid LCNEC area accounted for 25 % of it. The solid component contained extensive geographic

necrosis (Fig. 1c) and consisted of generally monotonous growth of medium- to large-sized atypical cells with occasional trabecular patterns and a Rosette-like cell arrangement (Fig. 1d, e). The cells contained hyperchromatic nuclei with frequent mitotic figures. The tumor cells were positive for synaptophysin (Fig. 1f), NSE and p53, and negative for chromogranin, estrogen receptor (ER) and CD56. Synaptophysin was positive in 20 % of the LCNEC part, and NSE was positive in 50 % of it. Both were negative in the EC part.

As controls, we used normal pancreas and colon tissue in the immunohistochemical analysis. The overall features were consistent with high-grade NE carcinoma of large cell type. Finally, we diagnosed the tumor to be FIGO Stage IB (pT1bN0M0), combined with G1 EC and LCNEC. The EC component accounted for 75 % of the tumor tissue and was identified in a region away from the cervix, while the LCNEC component accounted for 25 % of the tumor and was situated in an area close to the cervix.

Postoperative chemotherapy was refused by the patient. Serum NSE was elevated at 21 months after surgery, and computed tomography (CT) followed by a biopsy of the

Fig. 1 Pathological findings of the endometrial tumor. A solid component (*left upper half*) and a G1 endometrial carcinoma (EC) component (*right lower half*) were observed in the tumor (**a** at a higher magnification for EC). The solid component consisted of large

odd-looking tumor cells with occasional trabecular and Rosette-like structures (**b**, **c**, with *white arrowheads*). Immunostaining for synaptophysin was positive in the solid component (**d**)

lymph node in the right mediastinum diagnosed metastasis from the uterine carcinoma. Synaptophysin was positive in 40 % of tumor cells. Palliative irradiation to the metastatic lymph nodes (LN) was performed. Thereafter, the patient developed brain metastasis, pleuritis and pericarditis carcinomatosa, and died of cancer at 51 months after surgery.

Gene mutations in the EC and LCNEC components

To investigate the histopathogenesis of the LCNEC component, we examined gene alterations in the mutation hotspots of 50 selected cancer-associated genes with the Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies Co., Carlsbad, CA). The mutation hotspots and interrogated 50 genes can be found at the following link: <http://www.thermofisher.com/>. Briefly, DNA was extracted from thinly sliced sections of formalin-fixed and paraffin-embedded tissues obtained from G1 EC, and LCNEC components, non-neoplastic endometrium (EM) and the metastatic lymph node, and subjected to the sequencing analyses. The precise methods of experiments are provided in the Supplementary Methods.

The EC and LCNEC components of the uterus shared identical alterations in *PTEN*, *PIK3CA* and *FGFR3*. The *PTEN* alteration was an almost homozygous missense single-base substitution (SBS), resulting in R130G (CGA to GGA). Similarly, *PIK3CA* was heterozygous for a missense SBS resulting in R88Q (CGA to CAA), and *FGFR3* was heterozygous for a single-base deletion (c.2383delC), resulting in a frameshift that deleted the 11 C-terminal amino acids, instead of adding a novel polypeptide consisting of 23 amino acids (p.P799R fsX23, based on NM_000142 *FGFR3* sequence). Only the EC contained another missense heterozygous SBS on *FGFR3*, resulting in A376T (GCA to ACA). Both the EC and LCNEC components had heterozygous SBSs in *CTNNB1*, but the EC contained G34R (GGA to AGA) while the LCNEC contained T41A (ACC to GCC). In addition, the EC contained a different T41A (ACC to ATC) mutation in *CTNNB1* with a low allele frequency of less than 10 %. No

gene alterations were identified in non-neoplastic endometrial tissue in these analyses. The metastatic LN completely shared mutations with the LCNEC, that is, the *CTNNB1* T41A mutation was ACC to GCC, not ACC to ATC. The mutational profiles of tumors are summarized in Table 1 and are detailed in Supplementary Table 1.

Discussion

Neuroendocrine carcinomas of the female genital tract are extremely rare, and comprise fewer than 2 % of gynecological cancers. They include carcinoid tumors, small cell NE carcinoma and LCNEC. NE features should be confirmed by positive immunohistochemical markers, such as chromogranin, synaptophysin, CD56 and NSE [4]. LCNECs, especially found in combination with other histologic types, have to be differentially diagnosed against dedifferentiated carcinomas. LCNECs are recognized by their arrangement in well demarcated nests, presence of trabeculae or cords with peripheral palisading, and positivity for staining of a neuroendocrine marker in more than 10 % of cells, as evident in this case. In dedifferentiated carcinomas, associations between cells are often accompanied by inflammatory cells that are loosely alveolar in structure, but in this case, cells were well clustered with few inflammatory cells. To the best of our knowledge, only 15 cases of endometrial LCNEC have been reported to date, including the present case (Table 2) [4–12]. Nine cases were pure LCNEC, and the remaining cases were associated with other types of tumors including EC (four cases), small cell NE carcinoma together with EC (one case) and papillary serous carcinoma (one case). Although the polypoid feature in small cell carcinomas of the endometrium is reported to be a reliable predictor of survival, this is not the case for LCNEC [7]. Although no established treatment has been demonstrated for LCNEC of the uterine body, the majority of cases are administered multimodality therapy. With regard to chemotherapy, patients generally received six cycles of cisplatin and etoposide [11].

Table 1 Summary of gene alterations found in the normal endometrium, large cell neuroendocrine carcinoma and lymph node metastasis

Gene name	Histological subtypes			
	EM	EC	LCNEC	LN
<i>PTEN</i>	N	R130G, ho	R130G, ho	R130G, ho
<i>PIK3CA</i>	N	R88Q, he	R88Q, he	R88Q, he
<i>FGFR3</i>	N	P799R fsX, he A376T, he	P799R fsX, he	P799R fsX, he
<i>CTNNB1</i>	N	G34R, he T41A ^a	T41A, he	T41A, he

Homozygous or heterozygous mutation was roughly estimated from the frequency of each mutated allele
N no change; *EM*, normal endometrium; *EC* endometrioid carcinoma; *LCNEC* large cell neuroendocrine carcinoma; *LN* lymph node metastasis; *ho* homozygous; *he* heterozygous

* Low allele frequency (4 %)

Table 2 Clinicpathological features of large cell neuroendocrine carcinoma (LCNEC) of the endometrium in the literature and in the present case

Case	Year	Author	Age	Surgery	Stage	Histology	Gross	Further treatment	Outcome
1 [5]	2004	Erhan	52	TAH and BSO	Ib	LCNEC	50 mm polypoid	RT and Cis + Eto	DOD (7 M)
2 [6]	2007	Mulvany	50	TAH, BSO, OMY and PPALND	IIIc	LCNEC	22 mm polyp	RT and Cis + Eto	AWD (12 M)
3 [6]	2007	Mulvany	80	TAH, BSO and PLND	Ic	LCNEC and G3 EC (50:50)	45 mm polypoid	NFT	DOD (5 M)
4 [6]	2007	Mulvany	77	TAH and BSO	IIB	LCNEC and G1 EC (90:10)	75 mm polyp	RT	DOD (23 M)
5 [6]	2007	Mulvany	79	TAH and BSO	IIIa	LCNEC and G3 EC (15:85)	No	RT	AWD (2 M)
6 [6]	2007	Mulvany	88	TAH, BSO and PLND	IIIc	LCNEC, G3 EC and SmCC (50:45:5)	50 mm polypoid	RT	AWD (1 M)
7 [7]	2008	Albores-Saaedraa	42	RH	Ic	LCNEC	45 mm polypoid	Cis + Eto	AWD (9 M)
8 [8]	2008	Poligua	59	mRH, BSO, OMY and PPALND	IIIB	LCNEC and papillary serous carcinoma	Unknown	RT, bradytherapy and chemotherapy(unknown)	AWD (5 M)
9 [4]	2010	Tearada	40	TAH, BSO, OMY and PPALND	Ib	LCNEC with sarcomatous change	30 mm polypoid	Chemotherapy(unknown)	AWD (16 M)
10 [9]	2011	Deodhar	70	TAH, BSO and OMY	Ib	LCNEC	35 mm polypoid	Cis + Eto	AWD (6 M)
11 [10]	2011	Shahibi	59	TAH, BSO, OMY, PPALND and APPY	IIIC2	LCNEC	Polypoid	RT and Carbo + Taxol, Doxil, Cis + Eto and Oct	DOD (12 M)
12 [11]	2012	Makihara	73	surgery was not performed	IVB	LCNEC (diagnosis of biopsy)	Unknown	NFT	DOD (5 W)
13 [11]	2012	Makihara	73	TAH, BSO, OMY and PPALND	IIIC1	LCNEC	Unknown	Cis + Iri	AWD (6 M)
14 [12]	2013	Nguyen	71	RH, BSO, PPALND	IVB	LCNEC	Polypoid	Cis + Eto and Oct	AWD (32 days)
15	2015	Present case	61	mRH, BSO, OMY and PLND	IB	LCNEC and G1 EC (25:75)	Polyp	RT	DOD(51 M)

TAH total abdominal hysterectomy; BSO bilateral salpingo-oophorectomy; OMY omentectomy; PPALND pelvic and paraaortic lymph node dissection; PLND pelvic and node dissection; RH radical hysterectomy; mRH modified radical hysterectomy; APPY appendectomy; RT radiotherapy; Cis Cisplatin; Eto etoposide; NFT no further treatment; Carbo carboplatin; Oct octreotide; Doxil pegylated doxorubicin; Iri irinotecan; DOD dead of disease; AWD alive with disease; M months

In the present study, the EC and LCNEC components shared identical alterations in *PTEN*, *PIK3CA* and *FGFR3*, although the EC component contained an additional missense mutation in *FGFR3*. The shared *PTEN* R130G mutation is an inactivating alteration of the encoded product. *PIK3CA* encodes the p110 α catalytic subunit of phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K α). The shared *PIK3CA* R88Q is a gain-of-function mutation located in the adaptor-binding domain (ABD) of PI3K α . Although more than 80 % of *PIK3CA* mutations are found in its helical and kinase domains in other tumors, EC is reported to have additional mutation hotspots including the ABD, as observed in the present case [13]. Moreover, in the same PI3 K–AKT pathway, simultaneous inactivation of PTEN and activation of PI3K α are frequently observed

in approximately 40 % of EC cases [13]. The heterozygous *FGFR3* p.P798R fsX23 mutation was also associated with the both histological components with uncertain significance at present. However, *CTNNB1*, which encodes β 1-catenin, a cadherin-associated membrane protein, showed an interesting mutation profile in the present case, whereby the EC contained a heterozygous G34R mutation while the LCNEC contained another heterozygous T41A mutation. Both of the missense mutations were previously reported in EC [14] but not in NE carcinomas (COSMIC database). T41A mutation is the most frequent *CTNNB1* mutation (940 out of 5579 (17 %) *CTNNB1* missense mutations, COSMIC) and its biological significance has been confirmed, while G34R is a rare mutation of less than 2 % and the significance of glycine (G) residue alteration to arginine

(R) is uncertain. Activation of β 1-catenin via A41T in the LCNEC could be stronger than that with G34R.

PTEN inactivation was identified in more than 80 % of EC cases with the highest rates [15] and is considered an early event in the tumorigenesis observed in endometrial intraepithelial neoplasia (EIN) or in earlier latent precancerous lesions [16]. Additional hormonal selection, microsatellite instability or mutational activation of *KRAS* or β 1-catenin are known events occurring in the stepwise progression to EC [17]. One could speculate that both the EC and LCNEC components of the present case developed according to the stepwise EC tumorigenesis including the common *PTEN* inactivation. Although the common *PIK3CA* R88Q mutation was shared by the EC and LCNEC components, the *CTNNB1* mutation was completely different between them. There is a possibility that the independent *CTNNB1* mutation determined the fate of the precursor lesion, but further analyses are needed to confirm this speculation. The mutational profile of the endometrial LCNEC is not yet known, but the profile of pulmonary LCNEC has been precisely investigated [18]. The profile is generally divided into small cell lung carcinoma (SCLC)-like and non-SCLC-like types. The SCLC-like type is characterized by simultaneous *TP53* and *RB1* inactivation. The non-SCLC type shows frequent *TP53* mutations without *RB1* mutation, instead of the characteristic mutations found in adenocarcinoma, squamous cell carcinoma or other types of tumors. Frequently mutated genes in pulmonary LCNEC are *TP53*, *RB1*, *KRAS*, *STK11*, *KEAP1*, *PTPR*, *NKX2-1*, *NTRK3*, *NOTCH1*, *NOTCH4* and *EPHA5*. Among these genes, *TP53*, *RB1*, *KRAS*, *STK11* and *NOTCH1* were also examined in the present study, but no mutations were found in these genes. The cue for the development of endometrial LCNEC may be different from the pulmonary counterpart. The common *FGFR3* frameshift mutation was shared between LCNEC and EC in the present case, but the significance is unknown. The metastatic lymph node of the bronchopulmonary region seemed to originate from the LCNEC component because of the mutational profile.

In summary, the mutational signature in this study raised a possibility that the EC and LCNEC components were derived from a common precursor lesion but further analyses are required to clarify this. Investigations of gene mutations in multiple cases of endometrial NE carcinoma are needed to elucidate the mechanisms involved in the development of this highly malignant histological subtype, and to identify targets for effective personalized treatments.

Conflict of interest All the authors state that they have no potential conflict of interest to disclose.

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