Pathol. Oncol. Res. (2012) 18:523–528
DOI 10.1007/s12253-011-9386-7

CASE REPORT

Two Cases of Low-Grade Endometriod Carcinoma Associated with Undifferentiated Carcinoma of the Uterus (Dedifferentiated Carcinoma): A Molecular Study

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Received: 10 September 2010 / Accepted: 9 March 2011 / Published online: 30 March 2011

Abstract Dedifferentiated carcinoma (DC) is an uterine neoplasm containing both low-grade endometrioid carcinoma (LGEC) and undifferentiated carcinoma (UC). DC is an aggressive tumour even when the UC component represents only 20% of the entire neoplasm. In this paper, two cases DCs at different stages of development, in 61- and 83-year-old women respectively were reported. In addition, in these uterine malignancies microsatellite instability (MSI) and loss of heterozygosity (LOH) were investigated in order to explain its aggressive behavior, in both components. Case #1 presented metastases at diagnosis, while case #2 was at a lower stage. LGEC component was invasive in case #1 and intramucous in case #2. In both cases, UC components were characterized by a high degree of instability, in accordance of its aggressive behaviour and its architectural heterogeneity. Further studies with more numerous cases are mandatory to confirm these data.

Keywords Endometrial undifferentiated carcinoma · Dedifferentiated carcinoma · Microsatellite Instability · Loss of heterozygosity

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Introduction

According to the definition of the World Health Organization (WHO), Endometrial Undifferentiated Carcinoma (EUC) "is an epithelial malignant tumor, which is too poorly differentiated to be placed in any other category of carcinomas" [1].

This subtype of endometrial carcinoma is considered rare with an incidence of 1 to 2% [1].

A comparison between clinicopathologic examinations of 16 examples of EUC with examples of endometrioid adenocarcinoma, FIGO 3 grade, in the series of Altrausi et al., has demonstrated that EUC has a poorer prognosis than G3 endometrioid carcinoma [2].

Thus it is very important to make a correct diagnosis of EUC.

On histological examination, EUC is characterized by a proliferation of medium-sized, monotonous epithelial cells, organized to form solid sheets without any specific pattern. Endometrioid adenocarcinoma with 3 grade, on the other hand, shows solid areas with well-demarcated trabeculae and cords. Neoplastic cells in this subtype of uterine carcinoma resemble elements of glandular areas [3].

Dedifferentiated carcinoma (DC) is an uterine neoplasm containing both low-grade endometrioid carcinoma (LGEC)

and undifferentiated carcinoma (UC). DC is an aggressive tumor even when the UC component represents only 20% of the entire neoplasm [4].

In this study, two cases of these uterine malignancies, at different stages of development, in 61- and 83-year-old women respectively, were investigated for microsatellite instability (MSI) and loss of heterozygosity (LOH) in order to explain the aggressive behavior.

Materials and Methods

The material consisted of hysterectomy specimens with bilateral salpingo-oophorectomy and pelvic lymphadenectomy of two patients with uterine malignant epithelial neoplasms.

One of two women died only a few months after diagnosis for metastatic disseminated disease (Case #1), in other case (Case #2), instead, the patient showed pelvic recurrence after one year from diagnosis.

The specimens were fixed in 10% neutral-buffered formalin for a routine light microscope examination. The samples were embedded in paraffin, then 3 μ sections were cut and stained with hematoxylin-eosin for pathological examination.

For molecular analysis, 4-μm-thick histological sections were stained with hematoxylin and were examined under a stereo-microscope. Neoplastic and normal areas were manually microdissected, using sterile scalpels. Solid and glandular components of the tumors were micro-dissected separately. A neoplastic cellularity of at least 80% was obtained for all tumor samples. DNA extraction and purification were performed using a commercial kit (QIAamp DNA FFPE Tissue kit, QIAgen Inc., Valencia, CA, USA).

Microsatellite instability (MSI) and loss of heterozygosity (LOH) were investigated using nine microsatellite markers, including those recommended in the original [5] or revised [6] National Cancer Institute (NCI) panel for MSI testing in colorectal cancer (Table 1). Microsatellite markers were PCR amplified from tumor and normal DNA using primers labeled with Beckman Coulter WellRED fluorescent dyes D3 or D4 (Beckman Coulter, Fullerton, CA, USA). For PCR amplification, 2 μl of DNA were combined in a 25 μl reaction mixture containing 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 200 μM of each dNTP (Promega, Madison, WI, USA), 0.4 μM of each primer, 1.5–2.0 mM $MgCl_2$, 1.25 U Taq polymerase (Promega). PCR amplifications were performed in an AB 2700 (Applied Biosystems, Foster City, CA, USA) thermal cycler for 35 cycles. Individual PCR products were run on an eight-capillary CEQ^{TM}8000 DNA sequencer (Beckman Coulter), as previously described. [7] CEQ^{TM}8000 Fragment Analysis software (Beckman Coulter) generated electrophoretic profiles in which alleles appeared as peaks, with area and height proportional to the concentration of PCR fragments.

Normal and tumor DNA were compared for changes in size and the height of allele peaks at each microsatellite marker. MSI was defined as any change in microsatellite

Table 1 Microsatellite analysis of the undifferentiated (solid) and glandular components of two cases of dedifferentiated carcinomas

Micro-satellite Marker[a]	Cytogenetic Localization[a]	Gene of interest	Case # 1		Case # 2	
			Solid component	Glandular component	Solid component	Glandular component
MYC-L1	1p34-35	L-MYC	–	–	+	–
BAT40	1p13.1	HSD3B1	+	+	+	–
D2S123	2p16		LOH$^+$	–	+	–
BAT26	2p16	hMSH2	+	+	+	–
D3S1621	3p21.2-14.2	RASSF1	LOH$^+$	+	❖	❖
D5S346	5q21-22	APC	+	+	LOH$^+$	–
D10S1765	10q23-24	PTEN	LOH$^+$	+	LOH$^+$	LOH
D17S250	17q11.2-q12		+	+	–	–
D18S58	18q22.3-q23		LOH	+	+	❖
Number of markers with alterations			**8**	**7**	**7**	**1**
MSI status			MSI +	MSI +	MSI +	MSI -

[a] Primer sequences and microsatellite marker localizations are available at the Genome Database (www.gdb.org) and PubMed UniSTS (http://www.ncbi.nlm.nih.gov) web sites

Microsatellites in **bold** are included in the original or revised National Cancer Institute (NCI) panel for microsatellite instability testing in colorectal cancer [6, 7]

MSI microsatellite instability, - absence of MSI or LOH, *LOH*$^+$ simultaneous presence of MSI and LOH at the same microsatellite marker, ❖ not informative, *LOH* loss of heterozygosity, + presence of MSI

length within a tumor, due to either insertion or deletion of repeating units, when compared with normal tissue [5]. MSI was characterized by novel allele appearance or by allele mobility shift. Heterozygosity, i.e. the presence of two distinct alleles in the normal tissue, is the prerequisite for evaluation of LOH. Disappearance or significant reduction of one allele in the tumor DNA, as compared to the ratio observed in normal DNA, was described as LOH. Peak height values produced by the Fragment Analysis software were used to calculate the following ratio: $(\text{lower allele/higher allele})_{TUM}/(\text{lower allele/higher allele})_{NORM}$. Ratio values below 0.6, reflecting an allelic imbalance of 40% or more, were considered as indicators of LOH [7].

Results

Macroscopic Findings

In both cases the surgical specimens consisted of uterus with attached adnexa. In case #1, the uterus was enlarged and measured cm 13×8×6.

The ovaries, measuring respectively cm 2.2×1.1× and cm 2×1×, were macroscopically unremarkable.

The endometrial cavity was enlarged and contained a soft grey-white tumor, measauring cm 5×6×4.

The lesion was located in the upper body and the fundus of the uterus. On sectioning, the myometrium was entirely

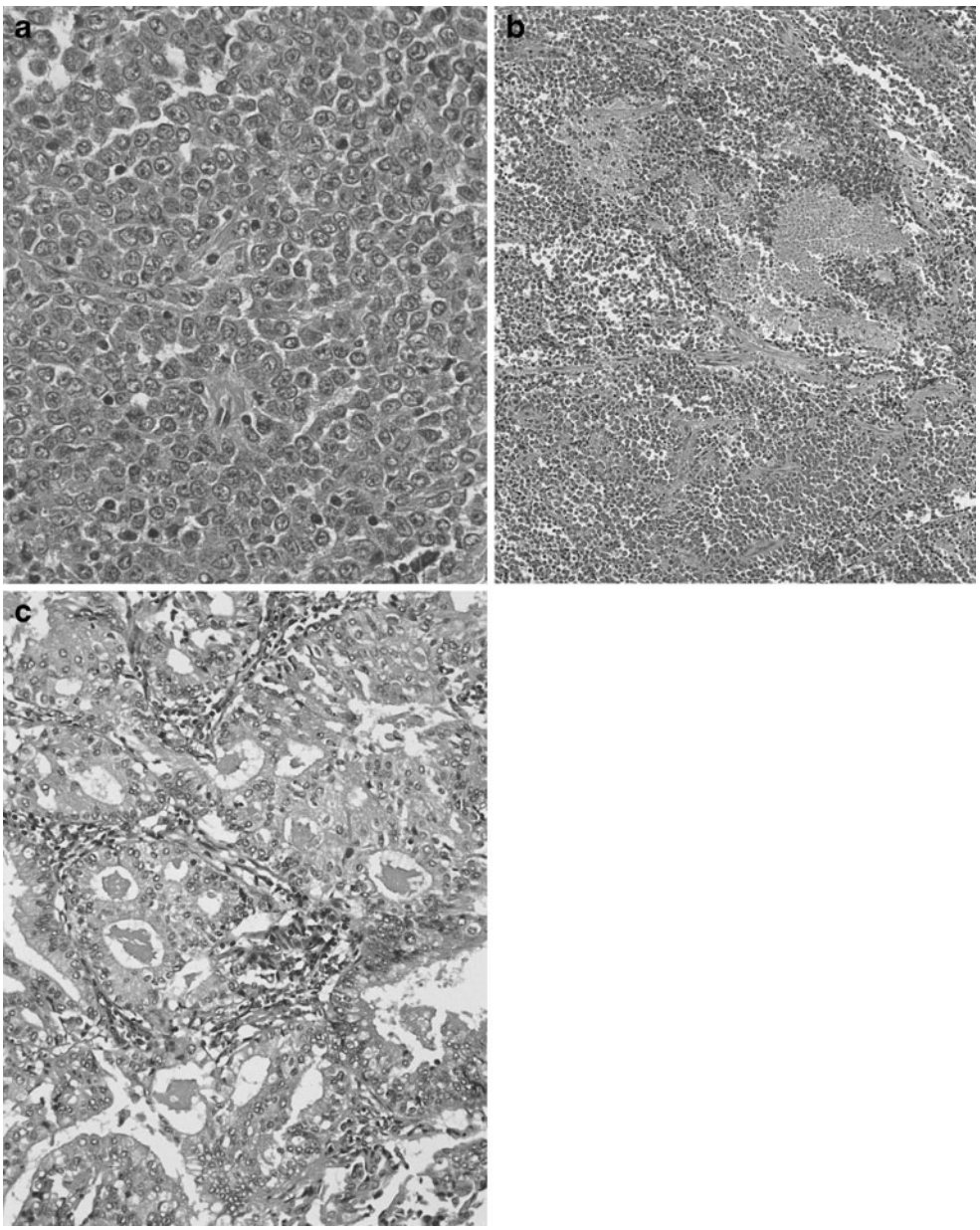

Fig. 1 On microscopic examination, both neoplasms were characterized by a proliferation of undifferentiated component (*solid component*), with medium-sized, monotonous epithelial cells organized to form solid sheets, without any specific pattern. The nuclei of undifferentiated areas (*solid component*) presented coarse chromatin, with evident basophilic nucleoli. Numerous mitotic figures were evident (**a**: x200). Necrosis was present in the remaining areas of both neoplasms (**b**: x 100). Neoplastic tissue showed the features of endometrioid adenocarcinoma, grade 2 in case # 1(**c**: x200)

replaced by white neoplastic tissue that showed large necrotic areas and extended to the serosa.

In case #2, the uterus measured 9×5×4 cm with unremarkable cervix, ovaries and serosal surface. A white neoplasm, with small necrotic areas, projected in the uterine cavity. On sectioning, this lesion had invaded 2/3 of the myometrium.

Microscopic Findings

On microscopic examination, both neoplasms were characterized by a proliferation of undifferentiated component (solid component), with medium-sized, monotonous epithelial cells organized to form solid sheets without any specific pattern

The nuclei of undifferentiated areas (solid component) presented coarse chromatin, sometimes with evident basophilic nucleoli**.** Numerous mitotic figures were evident (from 10 to 30×10 high power fields) (Fig. 1a). Necrosis was present in the remaining areas of both neoplasms (Fig. 1b).

In Case #1, only 15% of neoplastic tissue showed the features of endometrioid adenocarcinoma, grade 2 (LGEC) (Fig. 1c).

Both areas of undifferentiated (solid) and LGEC components had replaced myometrium and extended to the serosa and the cervix and were also present in the omentum.

Furthermore, in this case, the right pelvic nodes were metastatic. Thus, the stage of neoplasm in case #1 was III A, according to the FIGO system and $pT3_aN_1M_0$ according to the pTNM system.

Instead, in Case #2, the LGEC component was represented by only small intramucous foci (Fig. 2) while undifferentiated component had infiltrated more than half of the myometrium and the stage was IC according to the FIGO system and $pT1_cN_0M_0$ according to the TNM system.

Molecular Findings

Undifferentiated (Solid) Tumoral Components

In both cases, undifferentiated (solid) components were characterized by a high degree of instability. MSI was found at four loci in Case #1 and at five loci in Case #2. Additionally, LOH was observed at a single locus in case #1, while LOH and MSI occurred simultaneously at the same marker at three loci in Case #1and at two loci in Case #2. Overall, alterations were found in 8/9 markers in Case #1 and in 7 out of 8 informative markers in Case #2, therefore both solid components can be considered as unstable or MSI positive (MSI+) (Table 1).

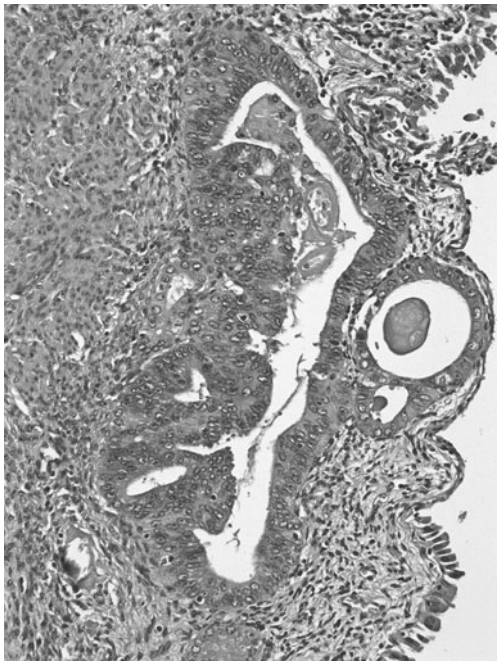

Fig. 2 Case #2, the LGEC component was represented by only small intramucous foci (x100)

Glandular Tumoral Components

Case #1 was characterized by a high level of instability in the glandular component too, as indicated by the finding of MSI at 7/9 loci investigated (MSI+), while LOH was absent. Conversely, the glandular component of Case #2 showed only a single LOH at D10S1765, therefore being considered as stable or MSI negative (MSI-) (Table 1)

Discussion

Endometrial carcinoma is the most common gynecologic malignancy in the United States; approximately 42,160 cases are diagnosed annually with 780 deaths [8].

Differences in epidemiology and prognosis suggest that there are two forms of endometrial cancer: those related to and those unrelated to estrogen stimulation [9]

Type I endometrial carcinoma is estrogen-related, usually presents histologically as a low grade endometrioid tumor, and is associated with atypical endometrial hyperplasia. These patients tend to have risk factors such as obesity, nulliparity, endogenous or exogenous estrogen excess, diabetes mellitus, and hypertension.

Type II endometrial carcinomas, unrelated to estrogen stimulation or endometrial hyperplasia, present with higher grade tumors or poor prognostic cell types, such as papillary serous or clear cell tumors. These maligancies affect often multiparous, and do not have an increased prevalence of

obesity, diabetes, or hypertension. The patients also tend to be older than women with endometrioid tumors [10].

While most serous (type II) cancers contain mutations of p53 [11], endometrioid (type I) adenocarcinomas show larger numbers of genetic changes, such as specific mutation of PTEN [12, 13], K-ras [14–16], Beta Catenin [17, 18] genes or microsatellite instability (MSI) [14, 19, 20].

Microsatellites are repeated sequences of DNA. The length of these microsatellites is highly variable from person to person. These repeated sequences are common, and normal.

In cells with mutations in DNA repair genes, some of these sequences accumulate errors and become longer or shorter. The appearance of abnormally long or short microsatellites in an individual's DNA is referred to as microsatellite instability (MSI) [21].

Microsatellite instability (MSI) is a form of genetic instability observed in virtually all tumors from patients with hereditary nonpolyposis colorectal cancer (HNPCC) [22] and in a subset of various sporadic tumors, including colorectal, gastric and endometrial cancer [23–25]. Defective DNA mismatch repair (MMR) gene is thought to promote tumorigenesis by accelerating the accumulation of mutations in oncogenes and tumor suppressor genes.

Loss of heterozygosity (LOH) in a cell represents the loss of normal function of one allele of a gene in which the other allele was already inactivated.

In oncology, loss of heterozygosity occurs when the remaining functional allele in a somatic cell of the offspring becomes inactivated by mutation. This results in no normal tumor suppressor being produced and this may result in tumorigenesis [26].

In this paper, two uterine DCs at different stages of development were investigated for microsatellite instability (MSI) and loss of heterozygosity (LOH), in order to explain their aggressive behavior.

Invasive LGEC component of case #1 showed high level of instability (MSI at 7/9 loci investigated), while intramucous LGEC of case #2 showed only a single alteration (LOH).

In both cases, UC components were characterized by a high degree of instability, with MSI at four loci in case #1 and at five loci in case #2. Moreover, LOH and MSI occurred simultaneously at the same marker at three loci in UC component of case #1 and at two loci in case #2.

These data can support other studies, which have demonstrated that high MSI in endometrial carcinomas likely is correlated with of higher histologic grade, endometriod type [27].

In addition, our investigation could confirm the more recent hypothesis, Shia et al., who suggested that DCs having an architectural heterogeneity, should be characterized by high MSI [28].

In addition, our cases demonstrated that genetic alterations in every component of DC are associated with defects in the mismatch repair pathway and these occur early in development of the neoplasm. However, the number of cases evaluated in our study are low and further studies, with more numerous cases, are mandatory to confirm our results

Moreover, because of a few data about previous familial history of the patients, we were unable to establish whether this high grade neoplasm arise in women with familial predisposition to develop the cancer.

However, because our investigation revealed that DCs have an aggressive behavior and can be considered as a malignancy with a poorer prognosis than G3 endometrioid carcinoma [2, 4], it is very important to make a correct diagnosis.

Acknowledgements The Authors wish to thank Professor Alex Gillan for the correction of the English language and Mrs Emilia Corradini for technical assistance.

Part of this paper was presented as a poster, during the SIAPEC-IAP Congress 7–9th September 2009 in Florence, Italy.

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